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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

PUBLIC HEALTH BULLETIN No. 43

APRIL, 1911

I. STUDIES UPON PLAGUE IN GROUND SQUIRRELS (IN FOUR PARTS)

PART I. Pathology and bacteriology of plague in ground squirrels

PART II. Notes on induced plague in ground squirrels

PART III. Immunity of certain squirrels to plague infection

PART IV. Insect transmission in relation to plague among ground squirrels

II. A PLAGUE-LIKE DISEASE OF RODENTS

BY

GEORGE W. McCOY

Passed Assistant Surgeon

PREPARED BY DIRECTION OF THE SURGEON GENERAL

WASHINGTON

GOVERNMENT PRINTING OFFICE

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STUDIES UPON PLAGUE IN GROUND SQUIRRELS.

[From the Federal laboratory, San Francisco, Cal.]

By GEORGE W. McCOY,
Passed Assistant Surgeon.

Part I.—PATHOLOGY AND BACTERIOLOGY OF PLAGUE IN GROUND SQUIRRELS.[1]

PATHOLOGY.

This report is based upon the examination of 246 naturally infected plague squirrels that have come under observation at the Federal laboratory during the past two years. Our experience with this number has given us fairly well defined ideas as to the appearance of these rodents when infected with this disease. It is probable that we have seen practically all of the pathological changes found in plague in ground squirrels and it is believed that the tabulations of the various lesions represent with reasonable accuracy their average frequency. The majority of the cases in this report have been verified by the isolation of the pest bacillus directly from the squirrel, or from a laboratory animal inoculated from a squirrel. A smaller number have been diagnosed upon the evidence furnished by the gross lesions in inoculated rodents, and upon the results of the examination of stained smears either from the naturally infected squirrel, or from the inoculated animal showing the characteristic lesions of plague. This evidence was regarded as sufficient only when other rodents from the same vicinity had been proven by a more complete examination to be infected.

The method of dissecting squirrels requires no special description. The peripheral lymphatic glands must be completely exposed. The abdominal and the thoracic cavities must be freely opened. Some of our assistants have become very rapid and proficient in dissecting these rodents, one of them having opened and examined over 800 in one day.

In the early part of the work, smears were made routinely from many squirrels that showed no lesions, but it was found that the results did not justify the time consumed. It may be stated that,

[1] In this bulletin the term squirrel or ground squirrel always refers to the California ground squirrel (*Citellus beecheyi*, Richardson).

as is generally true of other animals, man, rats, or guinea pigs, unless there is some pathological change distinguishable to the naked eye, it is unnecessary to examine microscopically smear preparations from the rodents.

GROSS LESIONS.

The study of the gross lesions of natural squirrel plague has been somewhat complicated by the fact that the majority of the animals we received had been shot. The traumatism often destroyed relations, and led to hemorrhages that obscured lesions. In other cases the decomposition changes that had occurred led to alterations of color for which certain allowances had to be made.

CLASSIFICATION.

As is true of almost any infectious disease, the division of cases into several classes is somewhat artificial, but it is convenient for purposes of study, and may be adopted profitably. It is customary to classify the lesions of plague in rodents into the acute, and the subacute or chronic. In studying plague in ground squirrels, a purulent bubo without any other lesion is found to be so common that it is convenient to regard these cases as forming a separate class. For the purpose of this report, we divide the lesions of the disease in squirrels into acute plague, subacute plague, and residual plague buboes.

The following table compiled from our records shows the frequency of the several classes of cases, together with the facts in regard to the way the animals were secured, the size, and the sex:

Type of cases.	How taken.				Size.					Sex.			Total.
	Shot.	Found dead.	Alive (died in captivity).	Trapped.	Grown.	Three-quarter grown.	Half grown.	One-quarter grown.	Not stated.	Male.	Female.	Not stated.	
Subacute	133	16	2	1	105	24	19	.....	4	89	55	8	152
Acute	50	9	.....	1	30	11	16	3	.....	35	22	3	60
Residual buboes	32	2	.....	....	22	3	6	.....	3	17	14	3	34
Total	215	27	2	2	157	38	41	3	7	141	91	14	246

LOCATION OF LYMPH GLANDS IN SQUIRRELS.

Before proceeding to a detailed consideration of the various types of cases, it is necessary to say a few words about the location of the lymphatic glands in squirrels. This is particularly important, as one or more of them will be found involved in about 84 per cent of all cases of plague.

The lymph glands in the neck are four or five in number on each side. They lie in a curved line around a large median structure, probably the thyroid. The axillary gland deserves no particular mention except the statement that it often rests in the center of a pinkish fatty mass which at first glance may be mistaken for diseased lymphatic tissue. The glands draining the posterior part of the body always require the most careful examination, as they are more frequently involved than those in the anterior part (axilla and cervical region). The lymph glands of the inguinal region and of the pelvis are found in three localities. The median inguinal is found near the midline just above the pubis. This structure is wanting in rats and in guinea pigs, but is constant in ground squirrels. Rather well back in the groin is found a chain of glands which often extends almost to the spinal column. We call these the posterior inguinal. The third set are those spoken of as pelvic glands. They are two in number, one on each side, and lie just at the bifurcation of the aorta. Analogous lymph nodes are found in other rodents.

The following table shows the frequency with which the several glands and groups of glands are involved in the various classes of cases:

Type of cases.	Total.	Location of bubo.					Multiple.	
		Cervical.	Axillary.	Median inguinal.	Posterior inguinal.	Pelvic.		
Acute	60	11	5	14	4	9	17	Axillary with inguinal 3 Cervical with inguinal 3 Multiple inguinal or inguinal with pelvic 11
Subacute	112	28	8	32	8	8	28	Bilateral axillary 1 Axillary with inguinal 1 Cervical with inguinal or pelvic 7 Multiple inguinal or inguinal with pelvic 19
Residual buboes	34	7	1	10	6	4	6	Cervical with inguinal 2 Multiple inguinal or inguinal with pelvic 4
Total with buboes	206	46	14	56	18	21		51

We have never seen a mesenteric bubo. In one case in which there was a cervical adenitis, a caseous gland was found just behind the sternum. The bubo in any of the types is frequently so large that it forms a distinct tumor mass readily recognized before an incision is made.

ACUTE PLAGUE.

We include here only those cases in which we found one or more hemorrhagic buboes without macroscopic necrotic foci in the internal organs. (Pl. I, fig. 1.) These constitute 24.4 per cent of the cases embraced

in this report. The gland is very commonly completely or partially surrounded by a bloody area. Occasionally a gelatinous exudate is found in the region. The contents of the bubo is dry, mealy, salmon colored, frankly bloody or blood tinged. A characteristic hemorrhagic gland may be present without any surrounding exudate. In a considerable number of cases the bubo will show a few points of blood-tinged pus, or the whole contents may be made up of grumous reddish purulent matter. Small subserous hemorrhages are found occasionally, particularly under the visceral pleura. Aside from a bubo, enlargement of the spleen was the most conspicuous lesion. This organ was usually deep red in color and quite firm in consistency. When decomposition had occurred it was soft and often slate colored. Occasionally it showed fine hemorrhagic points. It sometimes happened that we found buboes of different stages in the same animal; for example, a purulent cervical would be present along with caseous hemorrhagic inguinal glands.

SUBACUTE PLAGUE.

Under this head we have placed all cases in which there was a caseous bubo without evidence of surrounding hemorrhage, with or without macroscopic lesions of the internal organs; and those in which there were gross changes in the internal organs alone. The post-mortem findings varied greatly. A very few caseous granules in the spleen (Pl. I, fig. 2), or an abscess in the liver, sometimes constituted the only evidence of infection; on the other hand, we might have several necrotic glands and numerous large caseous foci in the liver, spleen, and lungs. The majority, however, presented a bubo along with foci in the spleen or the liver or both. This group contains 61.8 per cent of all cases considered; 73.7 per cent of them had buboes. The buboes were sometimes very large. It was not a very rare thing to encounter a cheesy gland 1.5 cm. in the long diameter. More frequently they were about 0.5 cm. through and contained a few necrotic points. Occasionally a pea-sized cheesy or purulent mass was found replacing part of the gland tissue. In some cases on account of numerous abscesses a honeycombed appearance resulted.

There was even more variation in the lesions in the internal organs than in the buboes. Cheesy or purulent foci were frequently found in the spleen, in the liver, in the lungs, or in two or more of these organs. The necrotic change was more commonly seen in the spleen than in the other organs. The nodules and the abscesses in the spleen may be very large. (Pl. II, figs. 3 and 4.) We have seen them almost as large as the last joint of one's little finger. In other cases numerous small purulent points may be found scattered throughout the organ. There was one case in which the only lesion was an

abscess about 1 cm. in diameter in the dome of the liver, the diaphragm being adherent over the surface of the organ.

Lungs.—We may have here either a pneumonic consolidation (Pl. III, fig. 5) usually rather grayish in color, or caseous or purulent areas (Pl. III, fig. 6; Pl. IV, fig. 7) similar to those found in the liver and the spleen. Caseous areas, especially the larger ones, sometimes appear to be in process of organization. This is true of the spleen and liver as well as of the lungs. In one case, a large abscess was found in the omentum, while in another, numerous small purulent foci were present.

Frequency of various lesions.—The following tabulation shows the frequency of lesions of the internal organs:

Organs involved.	Cases with—	
	Buboes and internal lesions.	Internal lesions only.
Spleen alone	17	9
Liver alone	6	8
Lungs alone	7	1
Spleen, liver, and lungs	6	3
Spleen and liver	26	17
Spleen and lungs	4	1
Liver and lungs	4	1
Total	70	40
Total cases spleen	53	30
Total cases liver	42	29
Total cases lungs	21	6

Adhesions.—Adhesions of serous surfaces were occasionally observed. When a lung was involved it was frequently adherent to the parietal pleura. When the liver was affected, the organ was often bound to the diaphragm. In a few cases the spleen was found to be adherent to the liver, to the abdominal wall, or to the omentum.

Jaundice was seen in several of the subacute cases, as well as in a few of the acute ones.

RESIDUAL BUBOES.

Under this head, we have considered those cases in which the only lesion was one or more purulent lymphatic glands. These constituted 13.8 per cent of all considered. Each one was verified by the inoculation of one or more laboratory animals. The glands were frankly purulent, the mass of pus usually being about as large as a pea (Pl. IV, fig. 8); occasionally it was much smaller than this, or the necrotic change was confined to a few points the size of a mustard seed. Sometimes the abscesses were very large, several being over 1 cm. in diameter. Quite often the process of softening was confined to a part of the gland, the remainder being fleshy looking, but without gross pathological changes. The pus was generally slightly yellowish, but never bloody.

The subacute cases were found at times in the same vicinity from which acute plague came; but occasionally they were received from sections of the country from which no other class of cases was secured. Probably it is largely a matter of accident that determines what type of squirrel plague is obtained from a given locality, as it is not likely that even in the districts where the most extensive shooting has been done we secure more than one out of every thousand infected animals.

GENERAL CONSIDERATIONS OF LESIONS.

When we compare the lesions of natural plague in the ground squirrel with those of the disease in other animals, the most striking feature is the extensive variations in the anatomical changes presented by the different examples of squirrel plague. In man, in rats, and in guinea pigs, the lesions in the great majority of cases are very uniform and constant. The subacute and chronic forms that constitute such a large percentage of the cases among squirrels are comparatively rare in other species. Squirrels are more closely related to the guinea pig than to most other rodents in which plague has been studied, but the guinea pig is notoriously susceptible and, as is well known, nearly always dies of acute plague when infected with a virulent culture. In a general way, we may say that lesions analogous to all of those observed in plague in squirrels may be seen in other animals, but that the percentage of types differs markedly. The acute type, which predominates in rats and in guinea pigs, is comparatively infrequent in squirrels, while the subacute type and residual lesions, so commonly seen in the latter, are relatively rare in the other rodents mentioned.

PLAGUE-LIKE DISEASE OF SQUIRRELS.

We have observed among squirrels an infection the lesions of which resemble plague very closely—so closely that even those who have had a large experience with both diseases may at times be unable to correctly judge as to whether the lesions in a given case are those of plague or of the plague-like disease. We may remark here that this disease causes plague-like lesions in the guinea pig and in certain other animals.

NEGATIVE CASES.

About 400 squirrels that were submitted to the inoculation test proved negative for plague. The cases fall into two classes: (1) Those which by bacteriological examination we were able to determine to be diseases other than plague; (2) those which we were unable to definitely diagnose. In the first class comes the above-mentioned plague-like disease and tuberculosis. The latter is very rare, but at least three cases have come under observation.

The second group comprises almost every conceivable lesion found in rodents. The fact was borne in mind that very little was known of the pathology of squirrel plague, and an animal presenting any abnormality that in the slightest degree aroused our suspicions was submitted to the inoculation test. The majority showed purulent lymph glands, many of which were microscopically indistinguishable from those we have called residual buboes. Others had abscesses in the viscera, especially in the spleen and the liver. Many of the rodents that had purulent lymph glands, with or without necrotic foci in the internal organs, were certainly examples of plague that had recovered. Such lesions are by no means common in squirrels, and the majority of the rodents in which they were found came from ranches and from localities that furnished infected animals.

Extensive adhesions of the liver to the diaphragm were very commonly met with in squirrels that had no other lesions. The animals almost always came from localities from which we had secured infected rodents.

SEASONAL PREVALENCE.

From the beginning of our work with plague infection in squirrels, the question has been kept before us as to whether there is any seasonal prevalence of the disease among these animals. It has been conclusively shown [1] that in certain parts of India there is a seasonal prevalence of rat plague, and that this coincides with epidemics of human plague. Without taking into consideration the distribution by months of cases of human plague of squirrel origin, which would introduce factors that need not be considered here, we may say that we have no reason for believing that squirrel plague is any more prevalent at one season of the year than at another. The figures for the cases by months have very marked variations, but a closer investigation shows that this is probably due to the changes in locations from which squirrels were sent. On one ranch that was found to be infected in June and July, 1909, infection was found again in January, 1910, and in June, 1910. We were inclined to believe that the disease prevailed most extensively during the summer until a heavy infection was found among the squirrels in Alameda County, Cal., in January 1910.

BACTERIOLOGY.

The bacteriology of squirrel plague does not differ materially from that of the disease in other animals. The organism, isolated either directly from a naturally infected squirrel or from a laboratory animal inoculated from a squirrel, is found to agree with the plague bacillus isolated from other sources. The evidence upon which the diagnosis

[1] Journal of Hygiene, Vol. VIII, No. 2, May, 1908, p. 266.

of squirrel plague is based was discussed somewhat fully in a previous paper.[1] It is sufficient to summarize this data by saying that a bacillus which is morphologically and culturally indistinguishable from *B. pestis* isolated from human beings and from rats in this country and abroad has been isolated from ground squirrels. This organism is pathogenic for the animals, guinea pigs, rats, mice, rabbits, and cats, which the pest bacillus is recognized as being capable of infecting; and does not affect certain animals, dogs and pigeons, that are known to be immune to *B. pestis;* furthermore, this organism has its pathogenic properties neutralized or diminished by the simultaneous injection of antipest serum made by the immunization of animals to foreign strains of the plague bacillus.

Theoretically it should have been possible to isolate the pest bacillus directly from the tissues of a large percentage of the infected squirrels. Practically this was not very often done, as the vast majority that were received had been shot at least 48 hours before they reached the laboratory. In most of them decomposition changes had begun, and in many were well advanced. While in some of these cases it would have been quite possible to have isolated the bacillus by cultural methods, the time consumed would have been greater than we could give to the work. It was a much more simple and certain procedure to inoculate a guinea pig or a white rat, and secure a culture from it. Another reason for the use of laboratory animals in this connection is the fact that while the cultural peculiarities of the pest bacillus are so well marked that the organism is not likely to be confused with any other, still in order to establish the diagnosis it is generally desirable to resort to inoculation experiments. It was just as satisfactory, and infinitely more convenient, to make the inoculation of a laboratory animal the first step in the procedure for identifying the organism as to make it the last one. We did not isolate *B. pestis* from all of the squirrels that were diagnosed and reported as plague infected. In a few cases when typical gross lesions were associated with characteristic organisms in smears, a diagnosis was made even if the effort to isolate the pest bacillus by cultural methods failed. In other cases the diagnosis was made when the gross lesions of plague were found at the post-mortem examination of an inoculated guinea pig or white rat, and when staining of smears from the tissues of the laboratory animal showed an abundance of typical bacilli. Cultures were nearly always made from the inoculated animals, but it occasionally happened that the growth was so badly contaminated that we failed to isolate the plague bacillus.

Unfortunately, it happened that frequently so large a number of suspected squirrels came to the laboratory on the same day as to make

[1] Public Health Reports, Vol. XXV, No. 2, Jan. 14, 1910, pp. 27-33.

it impracticable for want of time, and for lack of space in which to keep inoculated guinea pigs or white rats, to verify all of them by animal tests or by cultural studies.

MORPHOLOGICAL FINDINGS.

In the pathological classification of the lesions, those showing the presence of bacilli in smears were confined to two classes, the acute and the subacute. The lesions which were interpreted as representing convalescent stages of the disease were those that failed to show the bacilli in stained smear preparations or showed them in such small number as to be entitled to no weight in arriving at a diagnosis. The following table shows the frequency with which bacilli were found in cases in which the result of the microscopical examination was noted:

	Acute plague.	Subacute plague.
Present:		
Numerous	19	32
Few	12	13
Doubtful	5	17
Absent	6	74

Percentage of cases showing bacilli:	
Acute plague	71.4
Subacute plague	33.0

When bacilli were recorded as "numerous" they were so plentiful as to cover practically the whole field; by "few" we mean several characteristic bacilli in a field; by "doubtful" we refer to those cases which showed in smears a very small number of organisms morphologically resembling the plague bacillus. These were cases in which the microscopical findings were considered as negative in reaching a conclusion as to the condition present. For practical purposes they may be classed with those in which bacilli were absent. When only an occasional pest-like organism was found, as well as when none at all were present, the record was made to read "absent." This is justified by the fact that many bacteria are morphologically very likely to be mistaken for *B. pestis*, but they are almost invariably few in number compared to the number of plague bacilli usually found in smears.

The organisms are not always uniformly distributed throughout an affected tissue. Smears from different parts of a lesion may give different appearances. It happened several times that a smear made from a bubo was negative, while a second one from another part of the same gland presented a typical appearance. When characteristic bacilli were present they were not always found in all of the lesions. Thus, while they were more often present in the bubo than in the internal organs, it occasionally happened that they were found in great abundance in the viscera and were absent from the

bubo. One rather remarkable fact in connection with the examination of stained smear preparations was the occasional finding of cases that yielded the involution forms that are so frequently found in old cultures of the plague bacillus and are so characteristic of the growth on salt agar. By involution forms we mean, not the spherical (coccoid) forms which are found usually in considerable numbers in pest smears from almost any source, but the large spherical, flask-shaped, hourglass, and dumb-bell forms.

CULTURAL INVESTIGATIONS.

Cultures from the infected squirrels or from an inoculated animal were made on agar slants. The needle was drawn over the surface of the slant two or three times, and frequently a second (and occasionally a third) tube was streaked with the needle after the inoculation of the first one. In the majority of cases, a pure culture was obtained. Occasionally colonies were present that were obviously due to the growth of a contaminating organism. In these instances a colony of what was believed to be *B. pestis* was "fished" with an inoculating needle and transferred to a second tube. After a study of the growth, cultures were always made upon 3 per cent salt agar, in broth and in litmus milk. After 24 or 48 hours at 37° C. the salt agar culture was examined for the characteristic involution forms. The broth tube was kept until a delicate surface film and flocculi suspended in the media were observed. The reading of the litmus milk was made after an incubation of 2 or 3 days at 37° C. and a further period of a week or longer at room temperature. Litmus milk was used for the purpose of excluding the *Bacillus pseudotuberculosis rodentium*, Pfeiffer. It is well known that this organism not only resembles the pest bacillus morphologically and culturally, but also produces plague-like lesions in guinea pigs. We have constantly borne this in mind and have used the greatest care to prevent any confusion or error due to the presence of this micro-organism. One of the chief points of difference [1] between it and the pest bacillus is the reaction on litmus milk, *B. pestis* producing no change or a trifling degree of acidity, while *B. pseudotuberculosis rodentium*, Pfeiffer, renders it strongly alkaline. It is for this reason that in addition to the media usually employed in the identification of *B. pestis*, we have used litmus milk. The plague bacillus gives no characteristic reactions on media other than those mentioned, but as a matter of routine, cultures have occasionally been made on sugar broths, blood serum, potato, and gelatin. There was no gas formation in either glucose or lactose, though the former was rendered slightly acid. No visible growth appeared on potato. There was no liquefaction of gelatin.

[1] MacConkey; Journal of Hygiene, Vol. VIII, No. 3, June, 1908, p. 335.

In another part of this bulletin a plague-like disease in ground squirrels is discussed. A clear distinction is to be made between a plague-like disease and a pestis-like organism. While plague-like diseases have been observed, none of them has been found to be due to an organism resembling the plague bacillus, nor indeed has any such organism been found during the course of this work.

INOCULATION OF ANIMALS.

VIRULENCE FOR GUINEA PIGS AND WHITE RATS.

Guinea pigs and white rats are so susceptible to infection with *B. pestis* that they are indispensable in laboratory work with plague. We have used guinea pigs much more extensively than white rats as early in our work it was found that squirrel plague frequently failed to infect white rats when inoculated with the same material that was successfully used on guinea pigs. In one series [1] in which tissue from 11 squirrels was used to inoculate guinea pigs and white rats (one of each in each case), all of the guinea pigs died of plague while 2 of the 11 white rats remained well. In other words, had we depended for the diagnosis upon the inoculation of white rats, 2 of the 11 cases would have been missed. It is not believed that the relative immunity of white rats is due to any peculiarity of the pest bacilli in squirrels other than that of somewhat reduced virulence. It is well known that as a rule white rats are not so easily infected with *B. pestis* as are guinea pigs. Even guinea pigs are not uniformly susceptible; thus it has happened, though very rarely, that when two were inoculated from the same tissue, one would perish while the other either developed plague from which it recovered or escaped entirely. That an occasional guinea pig is immune is well demonstrated by a recent experience at the Federal laboratory. Two guinea pigs were inoculated with the same dose of plague culture; one died on the seventh day, while the other remained well.

In the inoculation of animals, we used such tissues as we thought were most likely to reproduce the disease. For example, if the bubo was a small, purulent one without bacilli in smears, while the spleen presented a nodule containing characteristic organisms, the latter organ was used. The following tabulations show the day of death of the guinea pigs inoculated directly from squirrels. For the sake of comparison figures are introduced showing the day of death of animals inoculated from cases of rat plague. All refer to vaccinated guinea pigs.

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[1] Journal of Infectious Diseases, Vol. VI, No. 5, November, 1909, pp. 676-686.

Length of life of animals inoculated with material from both squirrel and rat plague.

Day of death.	Squirrel plague—Federal laboratory.	Rat plague.		
		Bombay.[1]	San Francisco, Wherry, Walker & Howell.[2 3]	San Francisco, Federal laboratory.
Second	.....	3	.....	.....
Third	2	24	.....	.....
Fourth	15	23	.....	6
Fifth	15	26	.....	5
Sixth	22	18	.....	4
Seventh	11	10	.....	5
Eighth	7	2	.....	.....
Ninth	8	2	.....	.....
Tenth	2	2	.....	.....
Average duration of life.....days..	6.0	4.8	4.9	5.4

[1] Advisory committee appointed by the Secretary of State for India, The Royal Society, and the Lister Institute, Journal of Hygiene, Vol. VII, No. 3, 1907, p. 350.
[2] Time preceding death, 2 to 8 days.
[3] Wherry, Walker & Howell: Plague Among Rats in San Francisco, Journal American Medical Association, Vol. L, Apr. 11, 1908, pp. 1165–1167.

In the table we have avoided using figures where animals survived over 10 days. It will be seen that the guinea pigs vaccinated from cases of rat plague died on an average of about one day earlier than those vaccinated from cases of squirrel plague.

Length of life of animals inoculated with material from the several types of squirrel plague.

Day of death.	Guinea pigs vaccinated.			Guinea pigs inoculated subcutaneously.			White rats vaccinated.			White rats inoculated subcutaneously.		
	Acute cases.	Subacute cases.	Residual buboes.	Acute cases.	Subacute cases.	Residual buboes.	Acute cases.	Subacute cases.	Residual buboes.	Acute cases.	Subacute cases.	Residual buboes.
Second	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1	.....
Third	.....	2	.....	1	6	.....	.....	1	.....	.....	2	1
Fourth	8	7	.....	.....	13	2	.....	4	.....	.....	2	.....
Fifth	2	10	3	4	19	7	.....	2	.....	.....	1	.....
Sixth	5	12	5	2	22	7	.....	.....	.....	.....	.....	1
Seventh	4	6	1	1	14	2	.....	.....	.....	.....	.....	.....
Eighth	2	4	1	.....	7	4	.....	.....	.....	.....	.....	.....
Ninth	1	7	.....	1	3	2	.....	.....	.....	.....	.....	.....
Tenth	1	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....
Eleventh	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....
Twelfth	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Thirteenth	.....	2	.....	.....	2	1	.....	.....	.....	.....	.....	.....
Total animals used...	23	52	10	9	89	25	.....	7	.....	.....	6	2
Average duration of life.....days..	5.9	6.6	6	5.7	5.9	6.4	.....	4.1	.....	.....	3.5	4.5

A study of this table shows that there is practically no difference in the duration of life of guinea pigs inoculated from the various types of squirrel plague. In addition to the animals used in the above table it happened a few times that when guinea pigs were chloroformed on the fourteenth day they were found to be suffering from chronic plague.

SUSCEPTIBILITY OF MONKEYS TO INFECTION WITH THE BACILLUS FOUND IN GROUND SQUIRRELS.

Monkeys have usually been found to be quite susceptible to infection with plague. This refers to the strains derived from human cases and from rats. It seemed worth while determining the susceptibility of these animals to the cultures isolated from ground squirrels in California.

A monkey (Java) was vaccinated with a culture of *B. pestis* isolated about three months before directly from the heart's blood of a naturally infected squirrel. A guinea pig, a white rat, and a ground squirrel, all vaccinated, served as controls. The results are shown in the following table:

[Culture from squirrel 701, seventh generation on agar.]

Animals.	Day of death.	Lesions.
Monkey	Eighth	Acute plague.
White rat	Sixth	Do.
Guinea pig	Seventh.	Subacute plague.
Ground squirrel	...do	Do.

A pure culture of *B. pestis* was isolated from the spleen of the monkey.

SUSCEPTIBILITY OF WHITE MICE.

We have not conducted any experiments for the express purpose of learning the susceptibility of white mice to the squirrel strain of *B. pestis*. However, a few of these rodents have been used in immunity investigations. Four were vaccinated with a culture of the squirrel plague bacillus. Three died of acute plague; the fourth remained well.

SUSCEPTIBILITY OF WHITE RABBITS.

Six of these animals were vaccinated with 48-hour agar cultures of *B. pestis*, a culture isolated from a different naturally infected squirrel being used for each. Guinea pigs and white rats served as controls. The results are shown in the following table:

Day of death.

Culture No.	White rabbit.	White rat.	Guinea pig.
245/3	Fourth; acute plague	Seventh; acute plague	Remained well.
665/2	Sixth; acute plague	Fourth; acute plague	Ninth; killed; small purulent bubo.
71/3	Ninth; killed; caseous bubo.	Seventh; acute plague	Fifth; acute plague.
361/3	Seventh; acute plague	Ninth; killed; negative	Remained well.
72/3	Sixth; acute plague	Fourth; acute plague	Do.
396/3	Fifth; acute plague	Ninth; killed; negative	Tenth; subacute plague.

Judged by the results of this experiment, the white rabbits would appear to be more susceptible to the cultures used than are either white rats or guinea pigs. We have no adequate explanation to offer for the irregular results obtained here.

SUSCEPTIBILITY OF DOGS, CATS, AND PIGEONS.

Cats and dogs.—In the first experiment three young cats were used. One was vaccinated with a 48-hour-old broth culture isolated from a natural-plague squirrel. This animal remained well and when chloroformed 22 days after the inoculation showed no lesions. Two were given 2 c. c. of the same culture, one receiving the injection subcutaneously and the other intraperitoneally. Both died on the fifth day. The one receiving the culture subcutaneously had a large gelatinous and purulent exudate over the front of the belly, a caseous bubo, and an enlarged spleen. The other presented at post-mortem a thin purulent peritoneal exudate which covered the liver and the spleen in the form of a rather sticky film. A pure culture of *B. pestis* was isolated from the liver and from the heart's blood of each cat. A guinea-pig control vaccinated with the same culture died on the fifth day with the usual lesions of acute plague.

Experiment No. 2.—Three cats were vaccinated with the liver of a guinea pig dead on the fifth day after inoculation from a naturally infected plague squirrel. One died on the fifth day, presenting at autopsy a small caseo-purulent bubo, an enlarged spleen, and considerable pericardial effusion. A pure culture of *B. pestis* was isolated from the heart's blood and from the spleen. The others were chloroformed on the eighth day. Neither presented any lesions.

Experiment No. 3.—Two small dogs and three small cats were inoculated subcutaneously, each with 0.5 c. c. of a 48-hour broth culture of *B. pestis* of squirrel origin. The dogs were chloroformed on the fifth day. Neither presented any abnormality beyond a small œdema at the site of the inoculation. One of the cats died on the third, one on the fourth, and the other on the fifth day. All presented lesions of plague. A pure culture of *B. pestis* was isolated from those dying on the fourth and fifth days. A guinea-pig control, inoculated subcutaneously with 0.1 c. c. of the culture, died of acute plague on the fourth day.

Pigeons.—But one experiment has been conducted with pigeons. Two were inoculated subcutaneously with 2 c. c. of a 3-day-old broth culture of the plague bacillus isolated from a squirrel. Neither appeared to suffer any ill effects from the injection and they did not present any lesions when chloroformed 18 days after inoculation.

The experiments with cats, dogs, and pigeons, although limited in number, give results similar to those observed when other strains (human and rat) of *B. pestis* are employed.

CROSS IMMUNIZATION EXPERIMENTS.

The subject of plague immunity is one too large to be discussed in this bulletin. Those who are interested in this are referred to Strong's [1] exhaustive review of the subject. We have been concerned especially in trying to determine by cross immunization experiments the relation of the bacillus found in plague in squirrels to that from other sources, and the relation to *B. pseudotuberculosis rodentium*, Pfeiffer.

Immunization by a strain of B. pestis of human origin.—We had in our possession a culture of the pest bacillus that had been isolated about 10 years ago from a human case in San Francisco. This culture was almost entirely avirulent. Two guinea pigs were immunized by inoculating them subcutaneously with living 4-day-old cultures of this organism. Following the injection, the animals were sick for a few days and developed a rather extensive brawny induration over the front of the belly. This swelling disappeared within a week. Twenty-four days after the immunizing injection, the rodents were vaccinated with a virulent culture of *B. pestis* of squirrel origin. Both remained well, and when chloroformed 12 days later, presented no lesions. Three guinea pigs were inoculated for controlling the virulence of the test dose. Two of these died of acute plague, one on the fourth and the other on the sixth day. The third control remained well, probably an example of natural immunity to plague in the guinea pig.

Immunization by a strain of B. pestis from India.—In this experiment the immunizing agent was an avirulent culture of *B. pestis*, the history of which is not known, except that it came originally from Bombay. A quantity of this culture, equivalent to about one-third of an agar slant growth, was used to immunize each guinea pig. Eighteen days later a test dose was given by vaccinating the animal with a 48-hour agar culture of virulent squirrel plague. The results are shown in the following table:

Guinea pig.	Day of death.	Lesions.
Immunized	Remained well	
Do	do	
Do	do	
Control	Seventh	Subacute plague.
Do	Fourth	Acute plague.
Do	Seventh	Subacute plague.

A glance at this table shows that the foreign avirulent strain of the pest bacillus immunizes very effectually against *B. pestis* of squirrel origin.

[1] Philippine Journal of Science, Vol. II, No. 3, June, 1907.

Immunization by B. pseudotuberculosis rodentium, Pfeiffer.—As has been shown by several writers, the relation of this organism to *B. pestis* is in many respects a very close one. MacConkey [1] has reviewed the subject and added some experiments of his own. It is a most remarkable fact that this bacillus has the property of immunizing against *B. pestis*. One experiment of this nature is recorded here. Seven guinea pigs were inoculated with a live 48-hour-old broth culture of *B. pseudotuberculosis rodentium*, Pfeiffer, each being given 2 c. c. All of the animals were made sick for a few days, but soon regained their normal condition. Fifty-two days later, in order to test their immunity to the squirrel plague bacillus, they were vaccinated with a bubo from a guinea pig dead on the fifth day after vaccination from a naturally infected squirrel. One of the guinea pigs died; the remainder were chloroformed 70 days after the inoculation with plague. The results are shown in the following table:

Guinea pig.	Weight.	Lesions.	Remarks.
	Grams.		
No. 1	345	Large scar on belly; enlarged inguinal glands	
No. 2	235	Small purulent focus; left inguinal gland	
No. 3	190	None	
No. 4	170	do	
No. 5		do	
No. 6	540	Large scar on belly; enlarged inguinal gland	
No. 7	490	Had lesions of B. pseudotuberculosis rodentium. Cultures and smears negative, but a guinea pig inoculated subcutaneously with spleen was killed on seventeenth day and showed lesions of pseudotuberculosis rodentium.	Died fifty-first day after plague inoculation.
Control	350	Acute plague	Died sixth day.
Do	500	Subacute plague	Died eighth day.

In this experiment it will be seen that the previous inoculation very effectually immunized the guinea pigs against *B. pestis*, none of them showing any evidence of plague when examined, while the controls died of the disease.

A domestic strain of plague of human origin, a foreign strain, and *B. pseudotuberculosis rodentium*, Pfeiffer, all effectually immunized against the bacillus found in cases of plague in naturally infected ground squirrels.

SUMMARY.

The lesions of the various types of plague in ground squirrels, based upon the examination of 246 infected rodents, are described and the frequency of the several pathological changes stated. Especial attention is called to the predominance of the subacute type, and to the great variation in the lesions in the different cases.

Examination of stained smear preparations shows that in the majority of acute cases a probable diagnosis could be made by the micro-

[1] Journal of Hygiene, Vol. VIII, No. 3, June, 1908, p. 335.

scopical examination, while in most of the subacute and chronic cases this could not be done.

The bacilli present in squirrel plague would appear to be somewhat less virulent for guinea pigs than those from cases of rat plague in America and India. Guinea pigs, rats, mice, and rabbits are highly susceptible to the infection, cats moderately so; dogs and pigeons are probably immune, but the data available is scanty.

Cross immunization experiments demonstrated that avirulent cultures of *B. pestis* of domestic and of foreign origin, as well as a culture of *B. pseudotuberculosis rodentium*, Pfeiffer, immunize against the plague bacillus of squirrel origin.

Part II.—NOTES ON INDUCED PLAGUE IN GROUND SQUIRRELS.

These observations are based upon the examination of about 150 ground squirrels that have been inoculated in the course of various experiments conducted at the Federal plague laboratory, San Francisco, Cal., and at the branch laboratory at Oakland, Cal.

THE LESIONS OF EXPERIMENTAL PLAGUE.

When a squirrel is inoculated with a virulent culture of the plague bacillus, the pathological changes produced by the reaction of the tissues depend upon the susceptibility of the animal and, probably to a less extent, upon the dose and virulence of the culture used. If the animal is highly susceptible, death occurs on the third, fourth, or fifth day, and the lesions of acute plague are found at the autopsy. The post-mortem findings differ somewhat from those of acute plague in rats and in guinea pigs, but are analogous to the lesions seen in fatal cases in man when death occurs early.

If the squirrel is somewhat resistant but not entirely immune, lesions similar to those of subacute plague in other animals are found. In cases in which the rodent has offered enough resistance to the infection to have progressed rather far on the road to recovery, we have in the majority of instances a condition which for the sake of convenience we call "residual buboes." These cases present purulent foci in or at the site of the peripheral lymph glands. They may be compared to the purulent buboes seen in cases of plague in man when recovery is taking place. It is occasionally difficult to determine under which of these heads the lesions found in an animal should be classed, but as a rule one can determine this point without much trouble. The length of time that has elapsed between the inoculation and the death of the animal is not always a reliable indication of the lesions that will be found. It sometimes happens that a squirrel dying on the fourth or fifth day exhibits pathological changes that are as far advanced as those ordinarily found when death occurs on the seventh or eighth day. The separation of the lesions into these classes follows in general the divisions long recognized in plague in other animals, but on account of the frequency in the squirrel of the purulent lymphatic gland as the only evidence of infection it seemed well to make a separate class of the residual buboes. The classification of the lesions as those of acute plague,

subacute plague, and residual buboes has been found useful in the study of natural plague in squirrels. In over 400 naturally infected squirrels that have come under observation, we have seen duplicated all of the lesions that have been found in the experimentally infected animals.

ACUTE PLAGUE.

The term acute plague should be confined to the lesions that exist prior to the development of macroscopic necrotic foci in the internal organs. They are found when the animal dies between the third and the fifth or sixth day after infection. The post-mortem appearances are as follows:

Local reaction.—There is practically always an extensive lesion at the site of inoculation regardless of the mode of introducing the organism—that is to say, the reaction is present whether the infection is brought about by subcutaneous injection or by cutaneous inoculation (vaccination). The local reaction is generally in the form of a blood-stained slough surrounded by an elevated boundary of granulation tissue. The slough varies in size from 1 to 3 cm. in diameter or is even larger. When a section is made through one of these areas, it generally will be found that there is a hemorrhagic infiltration at the base and in the side walls of the lesion. At times the necrotic tissue has been detached and a deep ulcer is left. A rather extensive brawny thickening around the region of the slough is very common. The widespread gelatinous infiltration so commonly seen about the seat of inoculation in guinea pigs is not usually found in squirrels, though it occurs occasionally.

Occasionally the site of inoculation is marked by a rather firm nodule varying in size from a pea to the last joint of one's little finger. These nodules are somewhat caseous and are often hemorrhagic. In a few cases in which the animal dies on the third or fourth day, there may be no lesion beyond the reaction at the site of inoculation.

Subcutaneous injection.—This condition, so common in plague-infected rats, is rarely seen in squirrels.

The bubo.—One or more lymphatic glands will be found involved in practically all cases. The swelling caused by the enlarged lymph node can usually be detected before the region is incised. When an incision is made, the gland often will be found to be partially or completely surrounded by a bloody infiltration. A gelatinous exudate may or may not be present. In the majority of cases the gland is a little larger than a pea. The contents of the capsule is nearly always bloody and softened, and frequently, in addition to this, there is a more or less extensive purulent change. The pus may be present as a few mustard-seed-sized foci, or the whole of the gland may be converted into thick bloody pus. It may be said that in general

the tendency to purulent change is much more marked than is the case in either guinea pigs or rats.

Occasionally only one gland is involved; more often, a larger number. It is not very unusual to have both axillary and both inguinal lymph nodes converted into buboes. When the inguinal glands are affected, those at the brim of the pelvis are almost invariably involved. It may not be amiss to call attention here to the fact that in the ground squirrel there is a lymph gland lying just above the pubis and very near to the median line. There is no corresponding structure in either rats or guinea pigs.

The spleen.—Occasionally the organ is very little changed, but as a rule it is found to be enlarged to several times the normal size, firm, and rather dry. The normal lilac color is replaced by a deep cherry red. Small hemorrhagic points are common.

The liver.—This organ is unchanged except perhaps for some engorgement and enlargement.

The lungs.—In this type of cases the lungs may be normal, but very frequently subpleural hemorrhages are found. These extravasations may be mere points, or they may be 0.5 to 1 cm. in diameter.

Hemorrhages.—Subcutaneous hemorrhages not directly connected with the bubo are seen occasionally. Subpleural hemorrhages have been mentioned. Extravasations are occasionally found under the peritoneum covering the intestines in the omentum, or in the mesentery. Subpericardial hemorrhages are rather common, as are petechiæ on the surfaces of the kidneys and of the suprarenals.

SUBACUTE PLAGUE.

When a squirrel dies about the sixth day or later, the lesions are usually quite different from those that have been described under the head of acute plague.

Reaction at site.—The site of inoculation often shows no reaction beyond a small brownish crust. In other cases, an ulcer marks the site of the slough that has been thrown off. Occasionally there is a marked brawny thickening. A small, firm papule is at times the only mark left to show where the organism was introduced. A small subcutaneous abscess is found in a few cases.

The bubo.—The bubo is generally purulent or caseous, with but little or no evidence of hemorrhage into the gland or surrounding it.

In some cases there is no lesion beyond a bubo, but in the majority the internal organs are involved. They may be described as follows:

The spleen.—This organ is enlarged and presents caseous or purulent foci varying in size from a pin point to a mustard seed and in number from two or three up to so many that the spleen somewhat

resembles this organ in plague in the guinea pig. Sometimes caseous nodules as much as 1 cm. in diameter or larger are found. They may markedly distort the organ. In some cases the nodules seem to be in the process of organization.

The liver.—Granules and nodules similar to those found in the spleen are often encountered.

The lungs.—Granules and nodules like those found in the liver and in the spleen are often found. At times they are so numerous as to give the appearance of a fairly complete consolidation to the organ. The nodules are grayish red in color, and are surrounded by a well-defined deep-red zone. One remarkable example of complete consolidation of a lung has come under observation. In this case a squirrel was inoculated subcutaneously with a virulent culture of the pest bacillus. The animal died on the ninth day. At the post-mortem nothing was found at the site of inoculation beyond a small nodule. There was no bubo. The right lung, which was consolidated throughout, was grayish in color and showed the markings of the ribs on the pleural surface. The cut surface was firm, gray, and dry. There were two areas of softening in the consolidated organ, each one being about 1 cm. in diameter. Pleural exudate is rarely encountered. It may be serofibrinous, or fibrinous in character; the latter is always associated with gross lesions in the lungs.

RESIDUAL BUBOES.

It not infrequently happens that when a squirrel is killed two or three weeks or longer after inoculation, no lesions will be found beyond an enlarged lymph gland which contains yellow purulent foci, usually located just under the capsule. In other cases the site of the gland is marked by an abscess the size of a pea or larger. When these buboes are submitted to the inoculation test, they may or may not prove to contain virulent plague bacilli.

DISTRIBUTION OF BACILLI.

In practically all of the cases of acute plague there is a very heavy septicæmia, and organisms may be demonstrated in enormous numbers in the bubo, in the internal organs, and in the heart's blood. In the subacute cases, a septicæmia may or may not be present, but the bacilli are nearly always to be found in the bubo and in the lesions in the internal organs. In the cases where the only evidence of infection is a purulent bubo, with or without purulent foci in the internal organs, pest-like bacilli are rarely to be demonstrated in smears, although, as has been stated, some of these cases may be shown by the inoculation test to contain virulent bacilli.

SUMMARY.

The lesions of acute plague are an extensive necrosis at the site of inoculation, a hemorrhagic or caseous bubo, usually surrounded by a bloody exudate, and an enlarged spleen. Hemorrhages into the internal organs and into the subcutaneous tissue are common.

Subacute plague is indicated by the presence of a caseous or purulent bubo without much surrounding reaction. Granular or nodular lesions of the liver, spleen, and lungs may or may not be present.

Residual buboes are the result of an infection that is not sufficiently virulent to kill the animal.

DEVELOPMENT OF LESIONS.

This experiment was carried out at the suggestion of Passed Asst. Surg. W. C. Rucker, who thought that possibly certain early lesions of squirrel plague were overlooked by the laboratory assistants who dissected the rodents during the course of the investigation to determine the extent of squirrel plague in California.

Six squirrels were inoculated by the cutaneous method from the liver of a guinea pig dead on the fourth day after inoculation from a naturally infected squirrel. One was chloroformed after 30 hours and one every 24 hours thereafter. The object was to try to trace the development of the lesions. One of the squirrels died on the third day of plague, and one on the fourth day; consequently none were chloroformed on those days. The animal killed at the expiration of 30 hours showed nothing beyond a little dried serum at the site of inoculation, and would certainly have attracted no attention in the routine examination. It would perhaps be justifiable to speak of the disease as being in the stage of incubation. The squirrel killed on the next day (54 hours after inoculation) had a dried bloody scab at the site of inoculation; in addition, the spleen was enlarged. A few fine subpleural hemorrhages were present. There was no sign of a bubo. Unless the subpleural hemorrhages had attracted attention it is not at all likely that infection in this squirrel would have been detected during the routine examination. One of the squirrels died on the third day. It presented well-marked lesions—namely, a large bloody local reaction, hemorrhagic and caseating buboes, an enlarged spleen, and a blood-stained serous pleural effusion. This animal was pregnant, and the wall of the uterus was hemorrhagic, especially about the placental site. Another of the rodents died on the fourth day, and the lesions were similar to those found in the animal that had died on the preceding day, the only material difference being that the lesions were somewhat further advanced in caseation. The squirrel killed on the fifth day had large caseo-purulent buboes, and, in addition, the spleen was much enlarged and contained many fine

whitish granules. The animal killed on the sixth day had caseo-purulent buboes, but no other lesions.

In order to get a satisfactory picture of the progressive stages in the development of the various lesions, it would be necessary to inoculate a considerable number of rodents and kill several each day. In this way an average picture would probably be obtained.

SUMMARY.

The lesions of induced plague in the ground squirrel are not clearly distinguishable until the third day after inoculation. Distinct focal lesions may be found in the internal organs (spleen) as early as the fifth day.

INFLUENCE OF SIZE OF DOSE.

Two experiments have been performed for the purpose of ascertaining the influence of the size of the dose of culture injected.

The culture used in the first experiment was one that had been isolated about eight months before directly from the liver of a natural plague rat found in San Francisco. A 48-hour agar growth was used.

The results are shown in the following table:

Dose of culture.	Day of death.	
	Guinea pig.	Squirrel.
0.0001 loop	Fourth	Fourth.[1]
0.00001 loop	Sixth	Sixth.
0.000001 loop	Eighth	Eighth.

[1] Intraperitoneally.

The results would apparently indicate that the size of the dose had some influence upon the length of time the animal survived after the inoculation. I am inclined to believe, however, that no such inference should be drawn from it. The squirrel that received one ten-thousandth part of a loop was inadvertently inoculated intraperitoneally instead of subcutaneously, and it is a well-known fact that intraperitoneal inoculation of *B. pestis* leads to a fatal result much earlier than when a culture is injected subcutaneously.

So far as the guinea pigs are concerned, other and more extensive experiments do not lend any support to the view that the variations in dose in this case made any material difference. The loop used took up approximately 266,000,000 bacilli from an agar culture; therefore about 266 bacilli was the smallest number injected. It is needless to remark that large errors are possible in the making of suspensions of the plague bacillus.

In the following table the experiment was originally designed to determine the influence of the size of the dose upon the relative

immunity of certain squirrels, but it seems to show that in susceptible animals the size of the dose has no material influence.

In this experiment a 48-hour broth culture of squirrel origin was used.

Dose of broth culture.	Weight of squirrel.	Day of death.	Dose of broth culture.	Weight of squirrel.	Day of death.
c. c.	*Gms.*		*c. c.*	*Gms.*	
0.01	560	Fifth.	0.00001	540	Twenty-sixth; killed; no lesions.
.01	420	Seventh.			
.0001	360	Do.	.000001	360	Fifth.
.0001	640	Eighth.	.000001	510	Seventh.
.00001	350	Fifth.			

It will be seen that the size of dose within the limits used had no influence upon the results. By a coincidence it happened that the two animals receiving the smallest dose lived just the same length of time as those receiving the largest dose. One of the squirrels used in this experiment was immune.

SUMMARY.

Considerable variations in the dose of culture used have no influence upon the length of time required to kill susceptible squirrels.

SOURCE OF CULTURES.

When the next experiment was performed we had at our disposal four cultures of the pest bacillus, two of which came from plague squirrels and two from human cases of plague that were of squirrel origin. One squirrel culture and one of the human cultures were isolated from infections at Los Angeles, Cal., and the others from cases occurring in Contra Costa County, Cal.

In each case a 48-hour agar culture was used to vaccinate the animal. The cultures had all been isolated directly from the naturally infected animals with the exception of the Los Angeles squirrel, which had been passed through a guinea pig in the process of isolation. These are shown by name in the table:

[Figures after name of culture indicate generation on artificial media.]

Source of culture.	Day of death.	
	Guinea pigs.	Squirrels.
Los Angeles, Cal.:		
Human (5)	Fourth....	Fourth.
Squirrel (5)	.....do....	Sixth.
Contra Costa County, Cal.:		
Human (3)	Fifth.....	Fifth.
Squirrel (4)	Fourth....	Twelfth; killed; subacute plague.

SUMMARY.

The table indicates nothing more than that one of the squirrels used was more resistant than its fellows, and that cultures from plague cases (squirrel and human) in the southern part of the State (Los Angeles) where the infection was recent were as virulent as those from the northern part of the State (Contra Costa) where the disease had been present several years.

VIRULENCE OF BACILLUS PESTIS AFTER EXPERIMENTAL PASSAGE THROUGH GROUND SQUIRRELS.

These experiments were undertaken for the purpose of ascertaining whether there was any alteration in the virulence of the pest bacillus after passage through a series of ground squirrels. The question was of some practical importance, as it seemed probable that if any marked attenuation occurred the existence of plague among the ground squirrels of California assumed a different aspect from a public-health point of view than if full virulence was maintained. It has been claimed by some observers that passage of the pest bacillus through a series of rats leads to a reduction in virulence. Work along this line with the wild rats of San Francisco was complicated by the presence of a large percentage of immunity [1] to plague infection. I may say, however, that we have successfully carried the infection through five generations in wild San Francisco rats without apparent loss of virulence.

Technique.—Each series of squirrels was started with a pure culture of *B. pestis*, the origin of which is noted. When a squirrel died, the spleen or liver was used to inoculate another squirrel, and so on, until the end of the series. In nearly every case a guinea pig and two or more wild rats were inoculated in the same manner from the same material. The object of using the guinea pigs and wild rats was twofold: First, they served as controls for the virulence of the bacilli used to infect the squirrel; and second, we were able to learn if any change in virulence for these animals (guinea pigs and wild rats) was caused by the passage of the organism through the squirrels. The latter question is one of considerable importance, because if it should happen that passage through squirrels reduces the virulence of the organism for rats, squirrel plague would be of much less importance than if the organism remained fully virulent for rats. It is obvious that under natural conditions the strain of plague among squirrels must have passed through many more generations than we have found it practicable to carry it through experimentally.

The culture used for the first series was derived from a natural plague rat, taken in San Francisco, on July 28, 1908. The spleen of this rat was used to vaccinate a guinea pig. This guinea pig died on

[1] Journal of Infectious Diseases, Vol. VI, No. 3, pp. 289-295, June 12, 1909.

the sixth day after inoculation and a pure culture of *B. pestis* was isolated from its liver. The third generation of this culture on an artificial medium (agar) was used to inoculate a squirrel and a guinea pig.

The table shows the progress of the experiment. In every case all of the rodents (squirrels, guinea pigs, and rats) of any number were inoculated (vaccinated) from the squirrel of the preceding number. The inoculations were all made by shaving the skin of the belly so closely as to abrade the epithelium and leave a raw surface into which the culture or tissue was rubbed. The figures show the day of death of the various rodents.

SERIES NO. 1.

No.	Day of death.				
	Squirrel.	Guinea pig.	Rat No. 1.	Rat No. 2.	Rat No. 3.
1	Sixth	Sixth			
2	Fourth	Fourth	Fourth	Fourth	
3	Third	Sixth	Seventh	do	Sixth.
4	Fifth	Third	Third	Tenth; killed; no lesions.	Do.
5	Sixth	Fifth	Nineteenth; killed; no lesions.	Tenth; chronic plague.	
6	Fourth	do	Thirteenth; killed; no lesions.	Sixth	
7	do	Sixth	Twelfth; killed; no lesions.	Twelfth; killed; chronic plague.	
8	Fifth	Seventh	Tenth; killed; no lesions.	Eighth	
9	Third	do	Twenty-third; killed; no lesions.	Twenty-third; killed; no lesions.	
10	Fifth	Fifth	Seventeenth; killed; no lesions.	Fourth	
11	Fourth	do	Fifth	Seventh	Fifth.
12	do	Third; had early plague, also pneumonia.	Seventh	Fourth	Tenth; killed; chronic plague.
13	do	Third	Fifth	Fifth	Fifth.
14	Fifth	Fifth	Seventeenth; killed; no lesions.	do	Do.
15	do	do	Fourth	Seventh	Seventh.
16	Sixth	Seventh	do	Fourth	Fourth.
17	Fourth	Sixth	Eighth	Third	Do.
18	do	Eighth	Fifth	Fourth	Do.
19	Sixth	Fifth	Sixth	Seventh	Ninth.
20	Third	Fourth	Fourteenth; killed; no lesions.	Fourteenth; killed; chronic plague.	Sixth.
21	Fifth	Third	Fifth	Second	Fourth.
22	Sixth	Fourth	Fourth	Thirteenth; killed; no lesions.	Thirteenth; killed; no lesions.
23	do	Fifth	Sixth	Sixth	
24	Third	Fourth	Fourth	Ninth; killed; no lesions.	Ninth; killed; no lesions.
25	Seventh	Sixth	do		
26	Sixth	Seventh	do	Twelfth; killed; chronic plague.	Fifth.
27	Fourth	Fourth; killed; no lesions.	Eleventh; killed; no lesions.	Eleventh; killed; no lesions.	
28	do	Fourth	Thirteenth; killed; no lesions.	Fifth	Fourth.

Average duration of life of squirrels, 4.7 days.

A guinea pig was inoculated from the last squirrel (No. 28). It died in four days of acute plague. The experiment was begun November 28, 1908, and terminated April 10, 1909.

The second series was started with a culture of the plague bacillus isolated directly from the lung of the first natural plague squirrel found in California. It had never been passed through a laboratory animal. The third generation (24 hours old) on agar was used to start the experiment. The chain was maintained as was the first by inoculation (vaccination) from the spleen or liver of the squirrels. The culture was originally isolated on August 6, 1908, and the series was begun on February 5, 1909; therefore, the culture had been isolated about six months prior to the beginning of this work.

SERIES NO. 2.

No.	Day of death.				
	Squirrel.	Guinea pig.	Rat No. 1.	Rat No. 2.	Rat No. 3.
1	Sixth	Eighth			
2	Fourth	Fifth	Thirteenth; killed; chronic plague.	Fourth	Sixth.
3	Fifth	None used	Twelfth; killed; no lesions.	Fifth	Do.
4	Seventh	Fifth	Fourth	....do	Fifth.
5	Third	Sixth	Eleventh; killed; no lesions.	Eleventh; killed; no lesions.	Eleventh; killed; no lesions.
6[1]	Fourteenth; killed; no lesions.	Ninth	Sixth	Seventh	Sixth.
7	Sixth	None used	Fifth	Sixth	Seventh.
8	Fourth	Fourth	Fourth		
9	Seventh	....do	....do	Fourth	Fifth.
10	....do	None used	Fifth	Fifth	Fourteenth; killed; chronic plague.
11	....do	Fourth	Seventeenth; killed; no lesions.	Fourth	Sixth.
12	....do	None used	Tenth; killed; no lesions.	Fifth	
13	Fifth	Third; beginning plague, died of pneumonia.	Fourteenth; killed; no lesions.	Fourth	

[1] Probably immune.

Average duration of life of squirrels, 5.25 days.

The second series was interrupted by encountering an immune squirrel (No. 6). It was, however, carried forward by giving the chain a fresh start, using a culture derived from squirrel No. 4.

On one or two occasions in each series the squirrel died within 24 or 48 hours after the inoculation, and presumably not from plague. When this happened another squirrel was inoculated from the spleen (which had been preserved on ice) of the preceding number in the chain.

A glance at the tables will show that there was no alteration in the virulence of the infecting organism for either squirrels, guinea pigs, or rats (*Mus norvegicus*).

Such variations in the time of death as occurred were probably due to varying susceptibility of the animals. As was to be anticipated, a large percentage of the rats proved immune. This agrees with the results of extensive experiments made to determine this point.[1]

SUMMARY.

Passage of the *B. pestis* through a series of 28 squirrels led to no alteration in virulence for squirrels, guinea pigs, or wild rats.

[1] Journal of Infectious Diseases, Vol. VI, No. 3, June 12, 1909, pp. 289-295.

Part III.—IMMUNITY OF CERTAIN SQUIRRELS TO PLAGUE INFECTION.

During the first year that the writer worked with experimentally infected ground squirrels, all of the rodents used were caught either in the city of San Francisco or in San Mateo County. This county, as well as the city of San Francisco, is located on the peninsula of San Francisco, and, so far as is known, the squirrels in this section have never had plague among them. Several hundred have been killed and examined, but no infection has been found. Inoculation showed that these squirrels were highly susceptible to plague, as failure to succumb to the infection was very rare. It seemed desirable to determine whether squirrels from the zone of squirrel plague in Contra Costa County, which is on the mainland, were equally susceptible. The squirrels from the two counties are of the same species. A number of those that were used in the following experiments were submitted to Prof. Joseph Grinnell, of the department of mammalogy of the University of California, for classification, and he pronounced those from the two counties to be identical.

The squirrels from Contra Costa County were caught at various points within the area known to be infected. In each experiment several squirrels from each county were inoculated with the same culture, in the same manner, and so far as practicable with the same dose. Before being used all of the animals were kept in stock long enough to exclude natural plague. In several of the experiments guinea pigs and white rats were used as "controls."

Experiment No. 1.

In this experiment the animals were all inoculated by the cutaneous (vaccination) method, with one loopful of a suspension in broth of a 24-hour-old agar culture of *B. pestis*. This culture had been isolated directly from the heart's blood of a naturally infected ground squirrel about three months before the present experiment was carried out. The fourth generation on agar was used.

Animals.	Weight.	Day of death.
	Grams.	
Guinea pig (control)	400	Sixth; acute plague.[1]
White rat (control)		Fifth; acute plague.
San Mateo squirrel	445	Do.[1]
Do	600	Do.[1]
Contra Costa squirrel	360	Ninth; subacute plague.
Do	Small.	Tenth; subacute plague.[1]
Do	360	Fourteenth; killed; residual bubo.[2]
Do	467	Do.

[1] Culture of *B. pestis* isolated from animal.
[2] Purulent lymph gland resulting from plague infection.

Judged by its effect on the guinea pig and on the white rat, it will be seen that the culture was fairly virulent. The two San Mateo County squirrels died early with the lesions of acute plague, while of the four Contra Costa squirrels two died of subacute plague and two had abscesses in the region of the lymph glands.

Attention is called to the fact that the squirrels which proved resistant averaged considerably less in weight than those that succumbed.

Experiment No. 2.

In this experiment the culture was one that had been isolated directly from a plague rat about 14 months before this work was undertaken. One loopful of a 48-hour agar culture was used to vaccinate each of the animals. The results are as follows:

Animals.	Weight.	Day of death.
	Grams.	
Guinea pig (control)	365	Ninth; subacute plague.
San Mateo ground squirrel	433	Fourth; acute plague.
Do	542	Seventh; subacute plague.
Contra Costa ground squirrel	361	Third; acute plague.[1]
Do	210	Second; no lesions.

[1] Culture of *B. pestis* isolated from animal.

This experiment is reported here merely for the purpose of recording all in the series, although it complicates rather than aids in solving the problem we are dealing with. That the culture was probably of low virulence is shown by the fact that the guinea pig died on the ninth day. One of the Contra Costa squirrels died about 48 hours after the inoculation, but the cause of death could not be ascertained. The other Contra Costa squirrel died on the third day with the usual lesions of acute plague.

Experiment No. 3.

In this experiment the spleen of a guinea pig that had died of acute plague on the fifth day after inoculation with tissue from a naturally infected ground squirrel was used to vaccinate each of the animals mentioned in the following table. On account of a shortage of guinea pigs no control was inoculated.

Animals.	Weight.	Day of death.
	Grams.	
San Mateo squirrel	545	Sixth; acute plague.[1]
Do	445	Eleventh; subacute plague.[1]
Contra Costa squirrel	500	Eighteenth; killed; residual bubo.[2]
Do	442	Do.

[1] Culture of *B. pestis* isolated from animal.
[2] Purulent lymph gland resulting from plague infection.

The Contra Costa squirrels were apparently in good health when they were chloroformed, although at the post-mortem examination one of them showed an old lesion of plague.

Experiment No. 4.

The same culture was used as in experiment No. 2, but the eleventh generation was used instead of the sixth. One loopful of a broth suspension of a 24-hour agar growth was used to vaccinate the animals.

Animals.	Weight.	Day of death.
	Grams.	
Guinea pig (control)	325	Sixth; acute plague.
San Mateo squirrel	630	Seventh; subacute plague.[1]
Do	650	Fourteenth; killed; no lesions.
Contra Costa squirrel	360	Do.
Do	320	Do.

[1] Culture of *B. pestis* isolated from animal.

Here, for the first time in the series, we encountered an immune San Mateo squirrel; both of the Contra Costa squirrels were immune to the culture used.

Experiment No. 5.

The preceding experiments were conducted with strains of the plague bacillus that had been isolated in California. It seemed wise to test a series of animals using a strain from a different source. Therefore, in the present experiment, the animals were all inoculated by the subcutaneous method with a broth suspension of a plague culture from the stock of the Hygienic Laboratory, Washington, D. C. This culture is known as "Jedda," and has been carried for several years upon artificial media. Each of the animals was given subcutaneously 0.5 c. c. of a suspension of a 48-hour agar culture.

The results, which are very similar to those obtained with what might be called native strains of the plague bacillus, are shown in the following table:

Animals.	Weight.	Day of death.
	Grams.	
Guinea pig (control)	380	Sixth; acute plague.
White rat (control)	.....	Fourth; acute plague.
San Mateo squirrel	510	Do.[1]
Do	380	Seventh; subacute plague.[1]
Do	635	Ninth; subacute plague.
Contra Costa squirrel	780	Seventh; subacute plague.
Do	700	Eighteenth; killed; no lesions.
Do	560	Do.

[1] Culture of *B. pestis* isolated from animal.

In this case the dose of culture was very large, but in spite of this fact only one of the Contra Costa County squirrels died, while all of those from San Mateo County succumbed to the infection.

Experiment No. 6.

This experiment was designed to show the influence of the dose of culture on the immunity of the Contra Costa rodents as well as to compare their susceptibility with that of squirrels from outside the plague zone. Instead of using animals from San Mateo County, as had been done in the previous experiments, we used those from other counties (Tulare, Ventura) which have never furnished plague infection, although it has been carefully searched for. The culture used was a 48-hour broth growth planted from an agar culture that had been isolated a few months before from a naturally infected squirrel.

	0.01 c. c. culture.		0.0001 c. c. culture.	
	Weight.	Day of death.	Weight.	Day of death.
	Grams.		*Grams.*	
White rat	115	Third	135	Twenty-sixth; killed; negative.
Guinea pig	360	Seventh	.....	Eighth.
Do	400	.....do	410	Do.
Contra Costa squirrel	365	Thirteenth; killed; negative.	535	Seventeenth; killed; negative.
Tulare squirrel	560	Fifth	360	Seventh.
Ventura squirrel	420	Seventh	640	Eighth.

	0.00001 c. c. culture.		0.000001 c. c. culture.	
	Weight.	Day of death.	Weight.	Day of death.
	Grams.		*Grams.*	
White rat	110	Twenty-sixth; killed; negative.	170	Twenty-sixth; killed; negative.
Guinea pig	475	Thirty-fifth; killed; negative.	440	Seventh.
Do	390	Thirteenth	420	Thirteenth.
Contra Costa squirrel	700	Seventeenth; killed; negative.	550	Seventeenth; killed; negative.
Tulare squirrel	350	Fifth	360	Fifth.
Ventura squirrel	540	Twenty-sixth; killed; negative.	510	Seventh.

Here all of the Contra Costa squirrels survived while all but one of those from the other counties perished. But one of the white rats died. We have noted that the squirrel plague bacillus is sometimes only slightly virulent for white rats.

In order to determine approximately the number of bacilli in each dose, agar plates were made from each dilution and the number of colonies counted after 72 hours at 37° C. The results were: 0.01 c.c., innumerable; 0.0001 c.c., innumerable; 0.00001 c.c., 430 colonies; 0.000001 c.c., 42 colonies.

The cultures were made after vigorous shaking of the suspensions which were made in 0.8 per cent sodium chloride solution. It is not believed that the number of colonies that appeared indicates more than approximately the number of bacilli in the smaller doses.

DISCUSSION.

When we come to consider the significance of the results of these inoculations, we are confronted at once by the question as to whether the apparent immunity of the Contra Costa rodents may not have been merely a matter of accident. It is quite true that in experiment No. 2 the San Mateo squirrels lived longer than those from Contra Costa County, but on the other hand, in all of the other experiments, the majority of the Contra Costa squirrels outlived the San Mateo squirrels very materially. In fact, many of them were found entirely normal when chloroformed. It seems hardly likely that a coincidence should account for the fact. The results shown in the various tables may be conveniently summarized as follows:

	Total used.	Died.		Killed.	
		Acute plague.	Subacute plague.	Residual bubo.	No. lesions.
Contra Costa squirrels	16	1	3	3	9
San Mateo squirrels and others from plague-free zone	19	8	9	.....	2

Assuming that the Contra Costa squirrels are less susceptible to laboratory infection than are the San Mateo squirrels, we may for a moment consider what influence might account for this difference in susceptibility.

In the present case, the known fact that the squirrels in the one county (Contra Costa) have been exposed to plague infection for several years must be regarded as the cause of the relative immunity of the squirrels from that locality. This condition of increased resistance may have been brought about either by an acquired and possibly hereditarily transmitted immunity, or by the survival of animals that were naturally immune, and the descendants of such animals. At present we have no evidence bearing upon this point. It is not improbable that both factors play a part. It might be considered that the presence of the immunity was an indication of the dying out of the epizootic among the rodents of the infected county, but as we still find a large number of infected squirrels there, not much support is lent to this view.

We have suggested that this relative immunity of certain squirrels be put to practical use by regarding it as an index to the presence of

plague infection among these rodents even before actually infected animals have been found. The importance of this will be understood when it is explained that in the case of one county over 4,000 squirrels were examined before finding an infected animal. Now, if it can be shown that the squirrels from any county are highly resistant to the infection, as is the case with those from Contra Costa County, we would have fairly strong evidence that the rodents of that county had been the subject of an epizootic of plague. It is evident that the reverse is not necessarily true as highly susceptible squirrels might be present in part of a county if the infection had not reached the particular locality from which they were taken.

SUMMARY.

These experiments indicate that healthy squirrels from localities where the epizootic has prevailed for several years are decidedly more resistant to artificial infection with *B. pestis* than those from localities in which plague has not appeared among the squirrels.

Part IV.—INSECT TRANSMISSION IN RELATION TO PLAGUE AMONG GROUND SQUIRRELS.

PLAGUE BACILLI IN ECTO-PARASITES OF GROUND SQUIRRELS.

Several observers [1] have demonstrated the presence of *B. pestis* in ecto-parasites of man and of rats; therefore, it seemed desirable to determine the facts in regard to insects from the Sciuridæ.

The flea used in our work was *Ceratophyllus acutus*, Baker, the one most commonly found upon ground squirrels in California. The louse was *Hæmatopinus columbianus*, Osborn. For the identification of the lice I am indebted to Dr. L. O. Howard and Prof. Nathan Banks of the Bureau of Entomology, Department of Agriculture, Washington, D. C.

The evidence of the presence of *B. pestis* in the ecto-parasites under consideration rests upon two kinds of observations.

1. The examination of stained smear preparations made by crushing the parasite (louse or flea) and staining the films: The presence in such preparations of pest-like organisms of course would afford only presumptive evidence as to the nature of the infection. Previous workers [2] have noted that bacteria resembling *B. pestis* were not found in the stomachs of rat fleas other than those that carried pest bacilli. Our own observations made by crushing squirrel fleas give similar results. We have examined stained smear preparations from a large number of fleas taken from healthy squirrels, but have never encountered any organism that bore a close resemblance to *B. pestis*. In fact, they were generally free or almost free from bacteria of any sort. The only example we have of finding organisms in any number in crushed fleas other than those from plague animals was the following: A laboratory attendant captured a flea in the act of biting him on the forearm. The parasite which was identified as a *Ceratophyllus acutus* was crushed between slides. The stained smear showed a large number of organisms, some of which morphologically were quite like *B. pestis*. As we were working with plague-infected fleas and

[1] The reader is referred to Journal of Hygiene, Vol. VI, No. 4, 1906, p. 425, for a review of the subject, as well as for many experiments on insect transmission of plague.

[2] The advisory committee appointed by the Secretary of State for India, the Royal Society, and the Lister Institute, Journal of Hygiene, Vol. VII, No. 3, 1907, p. 398.

plague-infected squirrels, a searching inquiry was undertaken to ascertain the origin of the flea that bit this man. It was found that the only squirrel that he had handled on the day in question was one found dead in the stock cage. This rodent had a broken leg which was suppurating. Smears from the internal organs showed many bacteria, a few of which slightly resembled *B. pestis*. The animal showed none of the gross lesions of plague and bacteriological investigation confirmed the absence of the plague bacillus. At the time we examined the rodent no fleas were found on its body, but a number of lice were secured, crushed, and examined. The smears from these showed bacteria resembling those found in the flea that had bitten the man. While the evidence that the parasite found on the man had actually come from this squirrel is not conclusive, it is very probable that this was its origin.

In our experience lice taken from a healthy squirrel, even when kept several days before being crushed and stained, showed no organisms that would likely be mistaken for the plague bacillus.

2. The inoculation of a laboratory animal with feces from an insect, or with a part of the parasite's body: If the inoculated animal dies of plague, the evidence is conclusive that the pest bacillus was present in the feces or in the insect's body. It should be stated here that all of our work had been done with parasites derived from experimentally infected rodents.

The experiments of several workers [1] have shown that the feces of rat fleas are infected with plague bacilli, and our observations show that this is true for feces of squirrel fleas. Our work in this connection consisted of inoculation experiments alone; no microscopical examinations were made.

TECHNIQUE.

The following procedure was used: The flea or louse was crushed between two glass slides which were then separated. The one carrying the smaller film was stained and examined for pest-like bacilli, a note being made of the findings. The remnant of the insect on the other slide was gently removed with a small pledget of absorbent cotton moistened with normal salt solution and introduced into a pocket under the skin of a guinea pig.

When the fecal deposit was used for inoculation it was emulsified in a small volume of the saline solution and the suspension injected subcutaneously. The flea was transferred to a sterile test tube every day or two so as to separate the feces passed on different days.

[1] Journal of Hygiene, Vol. VI, No. 4, 1906, p. 425.

Plague bacilli in fleas.—In the following table is shown the results of the subcutaneous inoculation of guinea pigs with two squirrel fleas taken from a gray rat and three taken from a ground squirrel:

Flea No.	Sex of flea.	Species of flea.	Host.	Day of death of guinea pig.	Lesions in guinea pig inoculated with crushed flea.	B. pestis recovered from guinea pig.
1	Female	C. acutus	M. norvegicus	Sixth	Acute plague	Yes.
2	Male	do	do	do	do	Yes.
3	(?)	do	C. beecheyi	Eighth	Subacute plague	Yes.
4	Female	do	do	Eleventh	do	No.
5	do	do	do	Eighth	do	Yes.

Fleas 3, 4, and 5 had been removed from the host (squirrel) three days prior to the inoculation. Those from the rats, Nos. 1 and 2, were used immediately after removal from the rodent. In each case the stained film from the crushed insect showed a few pestlike organisms.

Plague bacilli in flea feces.—A squirrel flea (female *C. acutus*) taken from a plague squirrel was placed in a sterile test tube where during the ensuing few hours feces were deposited. A guinea pig inoculated with this material died on the sixth day and presented at autopsy the usual lesions of plague. A pure culture of *B. pestis* was isolated from the liver.

The following experiments were undertaken for the purpose of ascertaining if possible how long the feces of fleas remain infective. The insects were removed from a plague-infected squirrel soon after its death. The smears from the heart's blood of the rodent showed a heavy septicæmia. Feces passed by the fleas during various periods after removal from the host were used to inoculate guinea pigs.

Feces passed in 24 and 48 hour periods.

Flea No.	Guinea pig inoculated with feces passed in—			
	First 24 hours.	Second 24 hours.	Third and fourth 24 hours.	Fifth and sixth 24 hours.
1	Negative	Negative	Negative	Negative.
2	Death, ninth day; plague.	Death, eighth day; plague.	do	Do.
3	Negative	No feces	do	No feces.
4	Death, eighth day; plague.	do	do	Do.
5	Negative	Negative	do	Negative.

At the beginning of the above experiment two of the five fleas harbored the plague bacillus. After the second 24 hours all inoculations resulted negatively. The parasites were not fed during the period of observation.

Feces from fleas taken from another plague squirrel were collected at 24-hour intervals and used to inoculate guinea pigs. The results were as follows:

Flea No.	Guinea pig inoculated with feces passed in—			
	First 24 hours.	Second 24 hours.	Third 24 hours.	Fourth 24 hours.
1	No feces	Death, seventh day; plague.	No feces	No feces.
2	do	Death, eighth day; plague.	Death, fourth day; plague.	Death, tenth day; plague.
3	do	No feces	No feces	Death, eighth day; plague.

Two of the fleas harbored plague bacilli as late as the fourth day after removal from the host.

In both of these experiments the fleas were kept at room temperature.

Plague bacilli in lice.—A squirrel died of acute plague on the third day after inoculation. When examined a few hours after death, smears from the heart's blood showed a large number of characteristic bacilli. Six lice were removed from this animal; four were crushed at once, and all showed large numbers of characteristic pest-like bacilli. The remaining portion of each louse was inoculated into a guinea pig or a squirrel, with the following result:

Louse No.	Inoculated into—	Result.
1	Guinea pig	Died of acute plague on fourth day.
2	do	Died of acute plague on fifth day.
3	Ground squirrel	Died of acute plague on third day.
4	do	Died of acute plague on fifth day.

In every case the animals showed characteristic lesions of plague, and a pure culture of *B. pestis* was isolated, except from the guinea pig inoculated from louse No. 1.

Two of the lice were kept at room temperature until the fourth day, when one was found dead. Both were crushed and found to contain large numbers of pest-like bacilli. Parts of these lice were used to inoculate guinea pigs in the usual way (by the aid of a pledget of cotton), with the following result: Guinea pig inoculated from louse found dead, died of plague on the sixth day; that inoculated from louse killed, died of plague on the fifth day.

The following experiment indicates that a very large percentage of the lice harbor plague bacilli: Nineteen other lice from the same squirrel that furnished the insects in the preceding experiment were examined microscopically for pest-like bacilli four days after their removal from the host. Of 4 males, 2 showed such organisms and 2 were negative; of 15 females, all but 1 were positive.

A squirrel died of acute plague on the sixth day after inoculation. Twenty-nine lice were removed from this animal. Twenty-five of them were crushed and stained 24 hours after the removal from the host. Of this number, 20 showed pest-like bacilli, while 5 were negative. The remaining 4, which were put aside at room temperature, were found dead on the fourth day after their removal from the squirrel. These were used for inoculation experiments on guinea pigs. The rodents died of plague on the fifth, sixth, seventh, and tenth days, respectively, and a pure culture of *B. pestis* was isolated from each, with the exception of the one dead on the tenth day.

In another case a number of lice were taken from a squirrel which had died 4 days after inoculation; 17 were examined microscopically; 15 of them contained pest-like bacilli, while 2 showed no suspicious organisms. In this case a guinea pig inoculated from one of the crushed lice which showed bacilli died of acute plague.

The lice used for a series of inoculations to determine how long the parasites harbored virulent plague bacilli were removed from a squirrel dead of acute plague 6 days after infection. A smear from the heart's blood of this animal showed a large number of pest-like bacilli. Only those lice were used that showed characteristic organisms in stained preparations. Some of the lice examined on and after the sixth day showed objects which were interpreted as involution forms of the organism, similar to those found in salt agar growths. Indeed, the smears from some on the eighth and on the fourteenth days were noted as showing almost nothing but these forms; that is, practically no bipolar organisms were present.

The majority of the lice used were found dead upon the day of inoculation. All were kept at room temperature.

The results were as follows:

Time after removal of louse from host.	Louse killed or found dead.	Day of death of guinea pig inoculated with louse.	Lesions.
4 days	Killed	Eighth	Subacute plague.
Do	Found dead	Fifth	Acute plague.
6 days	Killed	Eighth	Subacute plague.
Do	Found dead	Fourth	Acute plague.
8 days	Killed	Fourteenth; killed	None.
Do	Found dead	Sixth	Acute plague.
11 days	do	Fifth	Do.
Do	do	Eleventh; killed	None.
14 days	do	Sixth	Acute plague.
Do	do	Fifth	Do.
19 days	do	Sixth	Do.
Do	do	Twentieth; killed	None.

NOTE.—The lice found dead may have died at any time subsequent to the last previous inoculation, as the containers were not examined in the interval.

Those lice used 19 days after removal from the host died after the fourteenth day; therefore this experiment demonstrates that lice from squirrels may harbor virulent plague bacilli for at least 2 weeks.

UNSUCCESSFUL ATTEMPTS AT INSECT TRANSMISSION.

On two occasions we have tried to transmit plague by removing a few fleas from a septicæmic squirrel and permitting them to feed upon a healthy one. The result was negative both times. The fleas were confined in a test tube and allowed to feed through the meshes of cheesecloth that covered the open end. This method had been used successfully by the workers in India [1] in experiments on rats and on guinea pigs. We may remark here that it is not especially difficult for a flea to escape through the meshes of the cheese cloth, and in our experiments this happened once or twice, but the insect was always recaptured.

We have made three attempts to transmit plague from one squirrel to another by putting lice from a plague animal showing septicæmia on a healthy one. In one case, 6 lice were used; in another, 11; and in the third experiment 44 were applied. The lice were placed among the hairs and near the skin. The result was negative in each case. In every instance control lice from the infected squirrel were crushed, and the resulting smears showed pest-like organisms. The parts of the controls used for inoculating guinea pigs caused plague in the rodents.

The conditions in such experiments are so highly artificial that the result of these attempts can hardly be accepted as negativing the possibility that lice may transmit plague infection.

FLEA TRANSMISSION.

The California ground squirrel almost always harbors a large number of fleas. The two species most commonly found are *Ceratophyllus acutus*, Baker, which has been used in all of our transmission work (except one experiment where rat fleas, *C. fasciatus*, were used), and *Hoplopsyllus anomalus*, Baker. The former is found much more frequently and usually in larger numbers. These experiments have been conducted in much the same manner as those by the earlier workers who have investigated the subject of flea transmission of plague. A rodent was inoculated with a virulent culture of the plague bacillus, or with tissue from an animal recently dead of the disease. Except in two cases, the strain used was of squirrel origin. Fleas were placed in the cage either at the time the rodent was infected, or after it sickened. After death, one or more healthy animals were placed in the cage with it, or fleas were picked from the dead rodent and placed in a clean cage with one or more healthy animals.

[1] Journal of Hygiene, Vol. VII, No. 3, 1907, p. 382.

In most of our experiments the cage used was an ordinary galvanized iron garbage can, 33 inches high by 19 inches in diameter. About 6 inches from the top a diaphragm made of ½-inch mesh galvanized-iron wire screening was set on four angle irons. When an infected squirrel or rat was placed in the cage, or when infected fleas were placed on healthy animals, the screen was wired to the angle irons to prevent any possibility of the rodents escaping. The portion of the inner surface of the can above the diaphragm was smeared with "tangle-foot" to prevent the escape of any fleas that might be inclined to wander up the sides of the can. When guinea pigs were used, a crock usually replaced the garbage can. A muslin covered wire screen served as a lid for the container. In addition to these precautions, the cage was placed in a galvanized iron tank, in the bottom of which was a thin layer of kerosene. This served to form a moat through which it was believed no parasite could pass.

From squirrel to squirrel by means of squirrel fleas (December 16, 1909).—A ground squirrel was inoculated subcutaneously with a broth culture of *B. pestis* derived from a human case of plague. This animal died on the fifth day. Three days before its death, 100 fleas, *C. acutus*, were put in the cage with it. While yet warm the dead rodent was removed from the cage, and 27 live fleas were taken from its body. Two of these were crushed, and staining of the resulting smears showed an abundance of pest-like bacilli in each. The 25 fleas remaining were put in a clean cage with a healthy squirrel. This animal died of subacute plague 10 days later, the buboes being in the region of the median, posterior inguinal, and pelvic glands. A pure culture of *B. pestis* was obtained from the liver. This experiment is conclusive in showing that *C. acutus* may convey plague from a sick to a healthy squirrel. It should be stated here that all of the squirrels were kept in quarantine for at least a month prior to their being used for the experiment. This was to exclude the use of any naturally infected ones. In fact all of these squirrels were obtained in a region in which no plague squirrels have ever been found.

From squirrel to guinea pig by means of squirrel fleas (November 23, 1909).—A healthy squirrel was inoculated subcutaneously with a culture of *B. pestis* obtained from the Hygienic Laboratory, Washington, D. C., that is, it was not a California strain of the organism. This animal died on the seventh day, and at necropsy presented the usual lesions of subacute plague in the ground squirrel. Three days before death occurred, 82 fleas (*C. acutus*) were introduced. Four guinea pigs were placed in the cage with the dead rodent and permitted to remain for half an hour, after which the squirrel was removed and dissected. The object was to permit the fleas to desert

the dead animal, and to go to the live ones. The fate of these guinea pigs is best shown by the following table:

Guinea pig No.	Day of death.	Lesions.	Location of bubo.
1	Fifth	Acute plague	Left inguinal.
2	do	do	Do.
3	Sixth	do	Multiple: 1 right inguinal, 1 neck.
4	Ninth	Subacute plague	Multiple: bilateral inguinal.

From squirrel to guinea pig by means of squirrel fleas (November 23, 1909).—This experiment was begun on the same day as the one immediately preceding, and the same culture was used to inoculate the squirrel. This animal died on the ninth day, and presented at autopsy the lesions of subacute plague. Four days before death, 89 fleas (*C. acutus*) were placed in the cage. When the squirrel succumbed, 4 guinea pigs were introduced and allowed to remain with the dead animal for half an hour. The fate of these guinea pigs is shown by the following table:

Guinea pig No.	Day of death.	Lesions.	Location of bubo.
1	Eighth	Subacute plague	None.
2	Twelfth	do	Right axilla.
3	Sixteenth; killed	None	
4	do	do	

From squirrel to white rats by means of squirrel fleas (March 24, 1910).—A squirrel which was vaccinated with the bubo of a guinea pig dead on the fifth day after inoculation from a naturally infected ground squirrel, died on the fifth day. Two days before its death, 220 fleas (*C. acutus*) were placed in the cage. After death 45 fleas were picked from the body and placed in a cage with 3 white rats. Two of these animals died of acute plague 5 days after the fleas had been put in with them. In one the bubo was in the neck; the other had two buboes, one in the axilla and one in the inguinal region. The third white rat was chloroformed two days after the death of his fellows and was found to be entirely healthy. In this case the evidence of infection of the white rat by means of squirrel fleas is conclusive, as there was no other way in which they might have acquired the disease.

From guinea pig to guinea pig by means of squirrel fleas (October 12, 1909).—A guinea pig was vaccinated with tissue from a guinea pig dead after inoculation from a case of natural squirrel plague. Four days later 100 fleas (*C. acutus*) were put in the cage. The animal

died on the seventh day after vaccination, 3 days after the fleas were introduced, and presented the lesions of acute plague. The rodent was allowed to remain in the cage for 24 hours after death in order to give the fleas ample time to leave the body. After it was removed, 2 fresh guinea pigs were introduced. One of the latter died on the sixth, the other on the eighth day. Both had the usual lesions of plague, the buboes being in the inguinal region. A pure culture of *B. pestis* was isolated from each animal.

From white rat to squirrels by means of rat fleas (April 12, 1910).—A white rat was vaccinated with a bubo from a white rat dead on the sixth day after inoculation with a culture of *B. pestis* of squirrel origin. Four days later 107 rat fleas (*C. fasciatus*, Bosc), which had been starved for 48 hours, were placed in the cage with this rodent. The animal died about 24 hours after the fleas were introduced. Fifty fleas were removed from the body and were placed in a clean cage with a healthy squirrel. This animal died of plague 10 days later, the bubo being in the inguinal region. About an hour after the removal of the dead white rat from the cage, a healthy squirrel was placed in it. This rodent was chloroformed while moribund on the seventh day, and found to be suffering from plague. The bubo was in the inguinal region. From this squirrel, as well as from the one infected by introducing the rat fleas into a clean cage, a culture of the plague bacillus was isolated.

The question might be raised that some of the animals believed to have been infected by fleas in reality had contracted the disease from mere contact with contaminated surroundings, other than insects, or by ingestion of feces from the sick rodents. We have frequently placed healthy guinea pigs in cages with plague-infected ones, but have never observed any spread of the disease if fleas were not present. This agrees with the results of extensive experiments conducted by the advisory committee appointed by the Secretary of State for India, the Royal Society, and the Lister Institute.[1]

In the two following experiments an infected animal was placed in a cage with 6 healthy ones, but without fleas, while in an adjacent cage the same conditions obtained, and in addition 100 squirrel fleas (*C. acutus*) were introduced. In each case the infecting agent was a culture of *B. pestis* of squirrel origin.

From guinea pig to guinea pig by means of squirrel fleas (November 11, 1910).—In this experiment, both inoculated guinea pigs died on the third day. The 6 healthy rodents in the cage without fleas

[1] Journal of Hygiene, Vol. VI, No. 4, May, 1906, p. 509.

remained well while 3 of those in the cage into which the fleas had been introduced died of plague, as shown in the following table:

Guinea pig No.	Day of death.[1]	Lesions.	Location of bubo.
1	Tenth	Acute plague	Inguinal.
2	Thirteenth	Subacute plague	Cervical.
3	Nineteenth	do	Inguinal.
4	Remained well		
5	do		
6	do		

[1] Counting from beginning of experiment.

From white rat to white rat by means of squirrel fleas (November 11, 1910).—An experiment identical with the preceding one was carried out with the exception that white rats were used instead of guinea pigs. The inoculated rat in the cage in which there were no fleas died on the third day. Upon removing the body, it was found to be partly devoured. Three of the "contacts" died of acute plague on the eighth day after the beginning of the experiment (i. e., 5 days after the death of the inoculated animal) all of the buboes being in the neck. The location of the buboes led to the conclusion that probably the infection had been acquired by feeding on the body of the inoculated rat. The importance of the location of the bubo is fully discussed by the advisory committee previously referred to.[1] The three rats that remained were chloroformed on the twelfth day after the beginning of the experiment and were found free from lesions. Whether the 3 that escaped infection were less cannibalistically inclined than their comrades we do not know.

With the rats that were in the cage with the fleas, the results were very different. In this case the inoculated animal died on the third day and its body had not been mutilated. All of the "contacts" died of plague in the order shown in the following table:

White rat No.	Day of death.[1]	Lesions.	Location of bubo.
1	Eighth	Acute plague	Axillary and inguinal.
2	do	do	None.
3	Twelfth	do	Inguinal.
4	Thirteenth	do	None.
5	do	do	Axillary.
6	Fourteenth	do	Inguinal.

[1] Counting from beginning of experiment.

After the death of the last one, 6 fresh white rats were put in the cage. Prior to introducing them the dead rat was surrounded by a wire screen, so that the new tenants could not touch the dead rodent, yet an opportunity was given for fleas to leave the dead

[1] Journal of Hygiene, Vol. VII, No. 3, 1907, p. 382.

animal for a living host. Three of these rats were found dead 5 days later. All had typical lesions of plague, the bubo in one being in the axilla, in another in the inguinal region, and the third had multiple buboes, one in the axilla and one in the inguinal region. The other 3 rats were chloroformed and found free from lesions.

SUMMARY.

Plague bacilli have been demonstrated in squirrel fleas (*Ceratophyllus acutus*, Baker) and in the feces from these insects by the inoculation test. The bacilli have been demonstrated in feces 4 days after removal of the fleas from the host.

Squirrel lice (*Hæmatopinus columbianus*, Osborn) taken from septicæmic plague squirrels often harbor the plague bacillus. The bacilli have been demonstrated in the lice at least 14 days after their removal from the host.

It has been shown beyond question that plague may be conveyed from one ground squirrel to another or from a ground squirrel to white rats by means of squirrel fleas (*C. acutus*) and from a white rat to a ground squirrel by means of rat fleas (*C. fasciatus*).

It has been shown, but perhaps less conclusively on account of the rather remote possibility of infection from contaminated surroundings other than fleas, that plague may be conveyed from squirrels to guinea pigs or to white rats, among guinea pigs, and among white rats by means of squirrel fleas (*C. acutus*).

A PLAGUE-LIKE DISEASE OF RODENTS.

[From the Federal laboratory, San Francisco, Cal.]

By GEORGE W. MCCOY, *Passed Assistant Surgeon.*

During the routine examination of ground squirrels we have encountered an infection the lesions of which are readily mistaken for those of plague. This disease also causes pathological changes in guinea pigs that are almost indistinguishable from those due to infection with *B. pestis* and occasionally produces plague-like lesions in rats and mice.

It is barely possible that we unknowingly have dealt with more than one disease entity, as no etiological agent has been discovered, and for a diagnosis we are compelled to rely upon the lesions produced in laboratory animals and the negative results of microscopical and cultural investigations. The post-mortem appearances in guinea pigs, where they are remarkably uniform and constant, afford the chief evidence upon which a diagnosis is based.

GEOGRAPHICAL DISTRIBUTION.

The disease has been observed in squirrels coming from many points. We have found it in those from nine counties, all lying between Los Angeles on the south and the Sacramento River on the north. The infection has been discovered in districts and even on ranches that have furnished plague squirrels as well as in those sections in which plague has not been demonstrated.

GROSS LESIONS.

GROUND SQUIRRELS NATURALLY INFECTED.

The following description is based upon the examination of 32 squirrels so infected:

Bubo.—The bubo is usually about the size of a pea, often larger, rather firm, and when cut presents a dry, yellowish, or blood-stained surface. Some hemorrhage is frequently found in the surrounding tissue. The gland structure is generally replaced by a firm caseous mass. Purulent glands have been seen, but are not common. In about 20 per cent of the cases a bubo was the only lesion observed.

Spleen.—When involved this organ is very much enlarged, perhaps to four or five times the normal size. It is fairly firm in consistency unless post-mortem changes have occurred. The color is generally

dark, almost black, or slate blue. Whitish or yellowish caseous granules varying in size from a pin's point to about 1 mm. in diameter, and in number from a dozen to so many as to be uncountable, are found throughout the tissue. They often project slightly above the surface of the organ. Pl. VI, fig. 11.

Liver.—Granules similar to those found in the spleen are frequently found in the liver, sometimes being more numerous than in the spleen. Quite often they are ashen gray in color.

Lungs.—These organs are very rarely involved, but in one case we have observed granules like those found in the liver and in the spleen. Occasionally the tissues are slightly jaundiced.

The following table shows the frequency of the various lesions in squirrels naturally infected:

Bubo:	
Present	29
Absent	3
Single—	
Inguinal	5
Axillary	4
Cervical	8
Pelvic	6
	23
Multiple—	
Inguinal and pelvic	4
Cervical and axillary	1
Cervical and inguinal	1
	6
Spleen granules:	
Present	24
Absent	8
Liver granules:	
Present	11
Absent	21
Lung granules:	
Present	1
Absent	31

INOCULATED SQUIRRELS.

As these animals were examined very soon after death, remarkably clear and distinct lesions were found. The only essential point of difference from the naturally infected squirrels was the presence of a local reaction at the site of inoculation. When the rodent had been infected by the cutaneous method (vaccination), there was a small scab and some thickening at the site, while occasionally a few papules were found. When the inoculation was by subcutaneous injection or

by implanting a piece of tissue in a subcutaneous pocket, there was a large purulent reaction, often hemorrhagic, occasionally gelatinous. The bloody exudate may extend to and surround the nearest lymph glands. Once or twice we have seen punctate hemorrhages in the lungs, and once a few granules.

Necrotic foci are constant in the liver and spleen of inoculated rodents dead of this disease, while in those naturally infected they are often absent. Probably this is due to the fact that often the latter are shot before the lesions have fully developed, while in the inoculated ones the disease is permitted to run its course.

INOCULATED GUINEA PIGS.

The lesions of the plague-like disease in these rodents are so similar to those of plague that while workers who have acquired a considerable degree of familiarity with both can usually differentiate them, yet cases are occasionally seen in which an error in the judgment of the gross lesions is made by the most experienced.

Reaction at the site of inoculation.—When a guinea pig has been vaccinated, there is a little thickening beneath the scab, often marked congestion, or a slightly hemorrhagic and occasionally gelatinous exudate. When the infecting material has been introduced beneath the skin, the reaction most commonly found is a dry, yellowish-white area resembling a membrane inserted between the muscles and the skin. Very rarely a large bloody exudate is seen.

Bubo.—When the inoculation is on the belly wall, the inguinal gland chains on both sides are usually enlarged and necrotic. (Pl. V, fig. 9.) The individual elements are about the size of a pea. The caseous matter is yellowish, dry, and sometimes hemorrhagic. A waxy appearance is quite characteristic. A bloody exudate surrounding the glands is rather frequent. The pelvic glands, those lying on each side of the midline just above the brim of the pelvis, are often involved when the primary bubo is in the inguinal region. In these cases fine pearly-white granules are usually seen following up the course of the great abdominal vessels. A bubo is a constant lesion, except in cases of intraperitoneal inoculation. It is present even in cases where the infection has been introduced through the nose (cervical) and in those where the disease has been induced by feeding (cervical or abdominal).

Spleen.—This organ is very large, friable, dark in color, and is almost always thickly studded with very distinct white granules up to 1 mm. in diameter (Pl. VII, fig. 13), which tend to vary in size more than do those in plague.

Liver.—Granules like those found in the spleen are very numerous although generally not so thickly set as in that organ. (Pl. VI, fig. 12.)

The liver sometimes shows irregularly shaped yellowish-green areas, the exact nature of which is not known.

Lungs.—Patchy congestion is common. Punctate hemorrhages are occasionally seen. Very rarely we see fine white granules similar to those in the liver and spleen. Serous pleural effusion has been observed on a few occasions.

In squirrels and in guinea pigs the lesions are remarkably constant. The animals always die of an acute infection, and there is very little variation in the findings in individual members of each species.

It is convenient to compare the lesions with those of plague as seen in ground squirrels and in guinea pigs by placing brief descriptions of the gross appearances of the two diseases in parallel columns. For the sake of completing the comparison the findings in smears and cultures are added to the table.

	Plague.	Plague-like disease.
Reaction at site, vaccination.	Brawny œdema with marked thickening.	Slight scab and moderate thickening.
Reaction at site, subcutaneous inoculation.	Large, œdematous, and purulent	Whitish membrane-like area, rarely œdematous.
Bubo	Surrounding exudate usually extensive; bubo dry, mealy, not waxy, often bloody.	Surrounding exudate less extensive; bubo waxy looking, pink, less frequently bloody.
Spleen	Enlarged; granules less distinct, usually more uniform in size.	Enlarged; very distinct discrete granules, often varying in size.
Liver	Granules usually absent or few in number.	Granules numerous and almost constantly present.
Lungs	Granular or nodular, lesions common.	Lesions rare.
Smears	Characteristic bacilli	Negative.
Cultures	*B. pestis* readily recovered	Do.

The table refers more particularly to the guinea pig, though it is to a large extent applicable also to the ground squirrel. In a general way it may be said that the lesions differ from those of plague in degree rather than in character.

PATHOLOGICAL HISTOLOGY OF THE PLAGUE-LIKE DISEASE.

This subject has been investigated only superficially. The remarks apply to guinea pigs and to ground squirrels.

Glands.—The glands involved show an extensive focal infiltration of round and polynuclear leucocytes. In some of the areas the nuclei show evidence of disintegration.

Spleen.—Aggregations of round and polynuclear cells are found scattered throughout the organ.

Liver.—The lesions may be demonstrated particularly well in this organ. The infiltration of new cells is often in the region where the bile duct, hepatic artery, and portal vein lie together. The areas made up of round and polynuclear cells replace the liver tissue for the most part, though sometimes the large vesicular nuclei of the liver cells may be seen lying well within an area of infiltration. The

centers of the foci often show a tendency to stain rather deeply with eosin. In other words, the lesions (in general) are those of a focal necrosis and the formation of minute abscesses.

PATHOGENICITY FOR RATS.

Mus norvegicus.—The experiments on rats have given rather irregular and to some extent contradictory results. It was believed at first that the lack of success in infecting *Mus norvegicus* (tame white and wild) offered a ready means of distinguishing this interesting disease from plague, though it was borne in mind that rats in general, even white ones, are not so uniformly susceptible to plague as are guinea pigs. This is especially true of the strain of *B. pestis* isolated from ground squirrels.

In the first case of this disease that came under observation, one rat was vaccinated and another inoculated from the spleen of a guinea pig dead on the sixth day after inoculation from a squirrel. Both of these rats were chloroformed on the seventh day. The vaccinated one was found to be entirely normal, while the one inoculated by the subcutaneous method had a small purulent reaction at the site, enlarged superficial lymph glands, and an enlarged dark spleen. The control guinea pig died on the seventh day with the usual lesions of the plague-like disease. Tissue from the second case was used to vaccinate a white rat. This rat died on the third day with lesions strongly suggestive of plague. A guinea pig vaccinated with the same tissue died on the seventh day with the usual lesions of the plague-like disease. In a third case, a white rat vaccinated directly from the spleen of a squirrel was found to be entirely normal when chloroformed on the fourteenth day, while the control guinea pig died on the sixth day with the usual lesions. These rather contradictory results led to the performing of several other experiments.

Variation of dose.—It appeared to be wise to try the influence of graduated doses of the infecting agent upon rats. Accordingly, the following series of inoculations was made, each animal being injected subcutaneously with an emulsion made from the liver of a guinea pig dead on the eighth day after inoculation:

Animal.	Size.	Dose.	Result.
Gray rat	Grown	2 c. c. of emulsion	Killed twenty-fourth day; no lesions.
Do	do	do	Died sixth day; lesions as below noted.
Do	do	1 c. c. of emulsion	Killed twenty-fourth day; no lesions.
Do	Half grown	0.1 c. c. of emulsion	Do.
Guinea pig (control)	Grown	do	Died on sixth day; usual lesions of plague-like disease.

The rat that died on the sixth day presented an enlarged mottled spleen, a liver studded with fine whitish granules, and a hemorrhage in the right axilla. The latter we thought might be traumatic. Cultures from the liver and from the heart's blood remained sterile, the usual result in this disease. The spleen of this rat was used to vaccinate a guinea pig, and as the latter remained entirely healthy, it is not possible to say whether the rat died of the disease under consideration. It will be shown later that it has been possible to demonstrate the presence of the disease in rats by the guinea-pig vaccination test.

Intraperitoneal inoculation of white rats.—One experiment was performed for the purpose of ascertaining the effect of intraperitoneal inoculation. An emulsion of liver tissue from a guinea pig dead on the sixth day was injected into the abdominal cavities of two half-grown white rats. Both of the rats died; one on the third and the other on the sixth day. There were no lesions in either case beyond an enlarged spleen, and it would have been impossible to have assigned any particular cause for the death of these animals but for the fact that spleen tissue from each was used to vaccinate a guinea pig, with the result that both guinea pigs died of the plague-like disease, one on the sixth and the other on the seventh day.

Rats sometimes die after a considerable time, and while they present no distinct lesions it can be shown that they harbor the infecting agent. This is demonstrated in the following experiment. Spleen emulsion from a guinea pig dead on the sixth day was used to inoculate the animals mentioned:

Animal.	Size.	Dose.	Result.
Gray rat	Half grown	1 c. c. emulsion, subcutaneously.	Died thirteenth day; no lesions, but spleen used to vaccinate a guinea pig which died on ninth day with usual lesions.
Do	do	2 c. c. emulsion, subcutaneously.	Died on fifteenth day; no lesions; guinea pig inoculated, negative.
Do	do	do	Died sixtieth day; no lesions; guinea pig inoculated, negative.
Do	do	0.1 c. c. emulsion, subcutaneously.	Died fourteenth day; no lesions.
Guinea pig (control)	440 grams	Vaccinated	Died sixth day; usual lesions.

When examined after death the first rat presented no lesions whatever, but the spleen used to vaccinate a guinea pig reproduced the disease in that rodent.

In another case a rat which died on the ninth day after subcutaneous inoculation had no lesions beyond an enlarged spleen, but the infection was reproduced in a guinea pig vaccinated from the rat's spleen.

In the following experiment the rats (wild *Mus norvegicus*) were apparently entirely refractory. Unfortunately guinea pigs were not

inoculated from them to determine whether they harbored the infection at the time they were killed. The infecting agent was spleen emulsion from a guinea pig dead on the seventh day after vaccination from a naturally infected squirrel.

Animal.	Weight.	Dose.	Result.
	Grams.		
Gray rat	230	1 c. c. spleen emulsion, subcutaneously.	Killed fourteenth day; enlarged spleen.
Do	265	1 c. c. spleen emulsion, subcutaneously.	Do.
Guinea pig	315	Vaccinated, spleen emulsion	Died sixth day; usual lesions.
Do	370	.....do.....	Do.

In addition to these experiments that have been recorded in detail we have inoculated a considerable number of white rats and gray rats. The great majority of these have proved immune to the infection.

Mus rattus.—One experiment has been performed with this species. Three of the rodents were inoculated with an emulsion made from the liver of a ground squirrel dead of the disease. One of the rats was vaccinated and two were inoculated subcutaneously. One of the latter died on the third day; the others remained well. A guinea pig (control) vaccinated with the same material died of the plague-like disease on the seventh day.

PATHOGENICITY FOR MICE.

With so low and uncertain a degree of virulence for rats it was thought that other members of the genus *Mus* would probably prove resistant, but attempts to infect mice negatived this view.

In the first experiment four gray mice were inoculated with the spleen of a guinea pig dead on the seventh day after vaccination from a squirrel. Two of the mice were infected by the subcutaneous method; one of these died on the third, the other on the fourth day. Two were inoculated by rubbing the spleen of the guinea pig on the shaved abdomen; one died on the fifth, the other on the sixth day. None of the mice presented any well-defined lesions. Two control guinea pigs died on the sixth day and presented the usual appearances of animals dead of the plague-like disease.

In the following experiment the dose was varied, and tissues from several of the mice were used to vaccinate guinea pigs for the purpose of ascertaining whether the mice had actually been infected with the disease (i. e., to exclude death from other causes).

The mice and the control guinea pig were inoculated subcutaneously with an emulsion made from the heart's blood of a guinea pig just dead of the disease.

Animal.	Dose.	Day of death.	Result of vaccination of guinea pig from spleen of dead mouse.
White mouse	Contaminated needle thrust under the skin.	Seventh	Dead sixth day; usual lesions of plague-like disease.
Do	do	do	No guinea pig used.
Do	0.1 c. c. emulsion, subcutaneously.	Fourth	Dead sixth day; usual lesions of plague-like disease.
Do	do	Sixth	Do.
Do	1 c. c. emulsion, subcutaneously.	Fourth	Do.
Do	do	do	No guinea pig used.
Guinea pig (control)	0.1 c. c. emulsion, subcutaneously.	Seventh	

The lesions in mice are not very well defined. In one or two instances lymph glands were enlarged, and one was probably caseous. The spleen was usually several times the normal size, and rather obscure granules were found in a few cases in this organ and in the liver. In several other experiments mice have been inoculated, and in every case have succumbed to the infection.

PATHOGENICITY FOR RABBITS.

Rabbits (Belgian hares) have been used in two experiments. One was vaccinated with spleen from a guinea pig dead on the seventh day. This rabbit died on the tenth day; the control ground squirrel (also vaccinated) died on the ninth day. In the second experiment a rabbit was inoculated subcutaneously with 1 c. c. of spleen emulsion from a guinea pig dead on the seventh day. This animal died on the ninth day. A control guinea pig given the same quantity of the emulsion died on the fifth day.

Lesions.—In the case of the vaccinated rabbit there was a small scab at the site; in the inoculated one there was a necrotic area about 2 cm. in diameter where the injection was made. Buboes were present in each case, the glands being about the size of a pea, and full of small yellowish-white points. The spleen and the liver of each animal were full of whitish granules, the largest being the size of a mustard seed. In the case of the rabbit dying on the tenth day, the omentum was filled with granules and nodules, and the diaphragm was studded with white granules. A white rat was inoculated in each case from the same material that was used to inoculate the rabbit and the control guinea pig, but with negative results.

PATHOGENICITY FOR GROUND SQUIRRELS.

As this disease was found in ground squirrels in nature, special interest attaches to the experimental reproduction of it in these rodents. It has not been feasible to use ground squirrels as extensively as we wished because they are difficult to obtain alive. For this reason the great bulk of our work has been done with guinea

pigs, and it is of course important to establish that the disease in these animals is the same as that found in the naturally infected squirrels. This we have done by inoculating squirrels from guinea pigs.

In the first experiment, a piece of liver from a guinea pig dead on the fifth day (second remove from naturally infected squirrel) was placed in a subcutaneous pocket in a ground squirrel. This animal died on the sixth day. The lesions were a purulent and hemorrhagic reaction at the site, a bloody exudate extending down over the thighs, and a yellowish caseous bubo. The spleen was full of white granules, the largest being perhaps 1 mm. in diameter. The liver contained granules similar to those in the spleen. There were punctate hemorrhages in the lungs. Here we have an example of the transmission of the disease by inoculation from a naturally infected squirrel to a guinea pig, through two generations in the guinea pig, and back into the squirrel. In order to carry the demonstration one step further, two guinea pigs were vaccinated from the spleen, and two inoculated subcutaneously with a small amount of heart's blood from the squirrel. These rodents all died with the usual lesions and in the usual time.

In another experiment, a ground squirrel was vaccinated with the spleen of a guinea pig (second remove in guinea pigs from a squirrel) dead on the seventh day. The squirrel died on the eighth day and presented lesions similar to those above described, and in addition a few whitish granules were found in the lungs. Ground squirrels have been infected in several other experiments that are of no special interest in this connection. No case of immunity to this disease has been observed. It is apparently more regularly fatal to these animals than is plague.

PATHOGENICITY FOR GOPHERS (THOMOMYS BOTTAE).

In order to determine the susceptibility of the gopher, two of these rodents which had been in stock for over a year were used. One was inoculated subcutaneously with 1 c. c. of spleen emulsion from a guinea pig dead of the plague-like disease on the sixth day, and the other was vaccinated with a loopful of the same emulsion. The gopher inoculated subcutaneously died on the third day and presented a small bubo, and an enlarged spleen which contained a number of minute whitish granules. The vaccinated gopher died on the fourth day with a bubo and granular lesions in the spleen. There was a bilateral clear serous pleural effusion in this case. That the second gopher died of the plague-like disease is proven by the fact that its spleen was used for the purpose of inoculating a guinea pig which died with typical lesions on the fifth day.

PATHOGENICITY FOR ANIMALS OTHER THAN RODENTS.

MONKEYS.

Two experiments have been made on monkeys. In the first, two animals were used, both being inoculated subcutaneously; one with 1 c. c. of an emulsion made from the liver of a guinea pig dead on the seventh day, the other with 0.5 c. c. of a salt solution suspension of the heart's blood of the same animal. Guinea pig controls inoculated with 0.1 c. c. of the liver emulsion and 1 c. c. of the heart's blood emulsion both died on the sixth day with the usual lesions of the plague-like disease. The first monkey (Java) perished on the eighth day, and the second (Rhesus) on the seventh day. In one case there was at the site of inoculation a whitish necrotic area such as has been mentioned under the head of lesions in the guinea pig; in the other, nothing was found to indicate the point where the infection was introduced. In each, a rather small caseous lymph gland was found, and the spleen appeared to be very much swollen. In one case, numerous fine granules were present in that organ. The other tissues appeared to be normal. Cultures from one monkey were sterile. None were made from the other. While the writer has no doubt but that the monkeys died of this disease, it should be stated that a guinea pig vaccinated from the liver of the monkey that died on the eighth day remained well. The liver had been kept in the ice chest several days before the guinea pig was inoculated.

In another experiment, a guinea pig, four white rats, and four white mice were used as controls for a monkey (Rhesus). All of the animals were vaccinated on the abdomen with the spleen of a guinea pig dead on the fourth day.

The results are shown in the following table:

Animal.	Weight.	Day of death.	Remarks.
	Grams.		
Guinea pig	315	Fifth	Characteristic lesions.
Monkey [1]		Eighth	Plague-like lesions.
White rat	70		Remained well.
Do	60		Do.
Do	60		Do.
Do	40		Do.
White mouse		Fourth	
Do		do	
Do		Fifth	
Do		do	

[1] This monkey had proven immune to vaccination with a virulent culture of the plague bacillus.

A white rat and a guinea pig were inoculated from the spleen of the monkey. The former remained well, while the latter died on the sixth day with the usual lesions of the plague-like disease.

The following is a brief description of the lesions noted in this monkey: A caseo-purulent bubo was found in the axilla, and another

in the inguinal region. There was but little surrounding infiltration. The spleen was much enlarged and contained many caseous granules, varying in size from a pin point to 2 mm. in diameter. The other organs were negative.

CATS AND DOGS.

But one attempt has been made to infect these animals. Varying doses of a spleen emulsion of a guinea pig dead on the fifth day were employed to inoculate them subcutaneously. Three young cats and two young dogs were used and none of them appeared to suffer any bad effects from the inoculation. All were carefully examined on the fourteenth day and found to be without any reaction at the site of inoculation or any indication of a bubo. A control guinea pig died on the sixth day with the usual lesions of the disease. It would seem safe to infer that cats and dogs are probably not susceptible to this infection.

PIGEONS.

The spleen of a guinea pig dead on the seventh day after inoculation from a naturally infected squirrel was used to inoculate subcutaneously two pigeons. The birds remained alive and apparently well, while two control guinea pigs died on the sixth day with the usual lesions. A second experiment with pigeons gave a like result.

The susceptibility of animals to this disease and to plague is shown in the following table:

Animals, etc.	Plague.	Plague-like disease.
Monkeys	Susceptible	Susceptible.
Cats	Moderately susceptible	Immune.
Dogs	Immune	Do.
Guinea pigs	Susceptible	Susceptible.
Rats	do	Usually immune.
Mice	do	Susceptible.
Rabbits	do	Do.
Ground squirrel	do	Do.
Gophers	Moderately susceptible	Do.
Pigeons	Immune	Immune.

SYMPTOMS.

As to the clinical manifestations of the disease, but little is to be said. When an infected guinea pig is examined on the third day or later, a reaction at the site of inoculation is found, but it is by no means so conspicuous as that found in plague. The animal refuses food, sits humped up; the coat is rough and the eyes are closed. When the end approaches, the animal lies on its side, gasps for breath, and slight spasmodic movements of the body and limbs occur.

We are acquainted with the difficulty in drawing any conclusions from temperature observations in guinea pigs, as the rectal temperature varies materially in apparently healthy rodents. Our efforts to

determine whether the disease is a febrile one were confined to two series of temperature observations. In each case the control was kept in the same cage with the sick animal.

	Temperature (degrees F.) of—	
	Infected guinea pig.	Control guinea pig.
First experiment.		
At inoculation	101.3	101.2
48 hours after inoculation	102.6	98.6
72 hours after inoculation	103.6	99.6
Dead next day.		
Second experiment.		
At inoculation	99.6	97.6
First day:		
Morning	99.2	97.4
Evening	101.8	97.2
Second day:		
Morning	102.6	97.0
Evening	105.0	97.2
Third day:		
Morning	103.8	96.6
Evening	104.6	96.8
Fourth day:		
Morning	104.6	96.5
Evening	[1]	[1]
Fifth day:		
Morning	104.0	96.8
Evening	103.4	98.6
Sixth day:		
Morning	98.0	97.0
Evening	[2]	.....

[1] Omitted. [2] Animal dead.

METHODS OF EXPERIMENTAL INOCULATION.

SUBCUTANEOUS INJECTION AND VACCINATION.

These methods have been so extensively used in the experiments recorded in the earlier part of this paper that it seems unnecessary to mention them further than to remark that both are certain and reliable methods of infecting susceptible animals.

In one experiment two guinea pigs of about equal weight were vaccinated with the spleen from a guinea pig dead on the seventh day after inoculation from a naturally infected squirrel. One was vaccinated in the ordinary way, the skin being slightly abraded in the shaving; while with the other, care was taken not to injure the skin. In each case the tissue was rubbed on firmly with forceps. Both died on the sixth day with the usual lesions. An unsuccessful attempt was made to infect a guinea pig by clipping the hair with scissors instead of using the barber's clippers, and smearing the infected tissue on the surface, not rubbing it in.

INTRAPERITONEAL INOCULATION.

We have used this method but once. An emulsion of tissue from a guinea pig dead on the seventh day was injected into the peritoneal cavity of a guinea pig. The inoculated animal died on the third

day. There was no evidence of peritonitis. The liver and spleen presented the usual granular lesions. Intraperitoneal inoculation of rats has been mentioned elsewhere.

NASAL INOCULATION.

It seemed worth while determining whether the introduction of the infecting agent through the respiratory passages would give rise to lesions in the lungs. A small pledget of cotton saturated with an emulsion of spleen from a guinea pig dead on the sixth day was placed in one of the nostrils of a guinea pig. This rodent died on the sixth day with the usual lesions, the bubo being in the neck. The lungs were negative. We thought that possibly some abrasion of the nasal mucous membrane had occurred through which infection had entered that led to the formation of the cervical bubo. A second experiment was undertaken in which a drop of a liver emulsion from a guinea pig dead on the eighth day was dropped into the nostril. This guinea pig died on the sixth day and at autopsy was found to have buboes in the neck and the other usual lesions of the disease. There were no changes in the lungs beyond a few hemorrhagic points such as are occasionally seen in guinea pigs inoculated in other ways. In neither case was there evidence of pneumonia or of focal lesions in the lungs.

FEEDING EXPERIMENTS.

Two guinea pigs were fed with chopped carrots mixed with blood and viscera from an artificially infected squirrel. One died on the seventh day after the feeding, and presented the usual lesions, the bubo being in the neck; the other remained well.

We have attempted to infect but one squirrel by feeding. The viscera of a squirrel dead on the ninth day after inoculation was fed to a squirrel. This animal died eight days later. The lesions were small caseous buboes in the neck, and numerous yellowish caseopurulent masses in the mesentery. The wall of the intestine contained a few thickened yellowish patches.

Attempts to infect rats by feeding have been uniformly unsuccessful.

NATURE OF INFECTION.

Having learned something about the susceptibility of the various animals, the modes of artificial infection, and the lesions in those to which we succeeded in communicating the disease, we will now present the results of experiments having to do with the nature of the infecting agent. Although we have not been able to cultivate any organism that we regarded as etiological, certain facts bearing upon the causative agent have been demonstrated.

The nature of the lesions, an involvement of a lymph gland or a chain of lymph glands draining the site of inoculation, with focal lesions in the viscera, would lead one to suppose that the disease was, at least in some stages, due to an organism present in the blood. Mention has been made in an earlier part of the paper of the successful inoculation of animals from the heart's blood of rodents dead of the disease. We shall record here experiments in which the heart's blood was diluted, and the dilutions injected into susceptible animals, thus enabling us to determine within rather wide limits, the degree of septicæmia present.

In the first experiment, the heart's blood of a guinea pig dead on the eighth day was diluted and used a few hours after death. Each animal received the dose indicated in the table suspended in 1 c. c. of physiological salt solution.

Animal.	Weight.	Dose.	Day of death.	Lesions.
	Grams.			
Guinea pig	260	0.01 c. c. heart's blood	Fifth	Characteristic.
Do	240	0.0001 c. c. heart's blood	do	Do.
Do	220	0.000001 c. c. heart's blood	Sixth	Do.
Do	330	0.00000001 c. c. heart's blood	Tenth	Do.

In this particular instance it would appear that 0.00000001 c. c. of the blood was sufficient to convey the disease. In the next experiment the heart's blood of the guinea pig dead on the tenth day (inoculated with 0.00000001 c. c.) was used to inoculate another series of guinea pigs. The results are shown in the following table:

Animal.	Weight.	Dose.	Day of death.	Lesions.
	Grams.			
Guinea pig	300	0.001 c. c. heart's blood	Fifth	Characteristic.
Do	350	0.0001 c. c. heart's blood	Seventh	Do.
Do	480	0.000001 c. c. heart's blood	Remained well	
Do	400	0.00000001 c. c. heart's blood	do	

In this case the animals receiving less than 0.0001 c. c. were unaffected.

These experiments clearly demonstrate that the disease is due to an organism that is present in large numbers in the heart's blood after death.

DURATION OF INFECTIVENESS OF TISSUES.

Tissues have been kept in the ice box, and after varying periods used to inoculate guinea pigs. The results have not been constant, probably because the conditions have not always been similar. On one occasion tissue retained its infecting power for 18 days; in

other cases it appeared to have died out by a sojourn of 4 or 5 days in the ice box.

At room temperature in one case infectivity remained for 6 days, at which time the last inoculation was made. Tissue of the same rodent dried in the incubator for 48 hours failed to convey the disease to a guinea pig.

THERMAL DEATH POINT.

Two experiments were performed for the purpose of determining the degree of heat necessary to kill the organism that causes the disease.

An emulsion of liver of a guinea pig dead on the eighth day was used. It was found that heating it in the Arnold Sterilizer at from 60° C. to 65° C. for 5 minutes has the effect of rendering it harmless, while the control animal inoculated with the same material unheated died on the fifth day with the usual lesions. In a second experiment an emulsion made from the spleen of a guinea pig dead on the seventh day was placed in small test tubes and heated in a water bath at the temperatures stated in the following table. The emulsion was then injected into the guniea pigs with the results shown below. One cubic centimeter was the amount heated and injected in each case. The tubes were plugged with cotton and the portion containing the fluid was immersed several inches below the surface of the warm water.

Animal.	Weight.	Degree of heat.	Heat applied.	Day of death.	Lesions.
	Grams.	*° C.*	*Min.*		
Guinea pig	255	45	10	Third	Characteristic.
Do	340	50	10	Fourth	Do.
Do	290	55	10	Fifth	Do.
Do	300	60	10	Remained well	
Do	270	65	10	do	
Guinea pig (control)	330	[1]		Fifth	Characteristic.

[1] Unheated.

The results of this experiment indicate a thermal death point about the same as that of most pathogenic bacteria.

EFFECT OF CERTAIN GERMICIDES.

A mixture of equal parts of salt-solution emulsion from the spleen of a guinea pig dead on the seventh day and glycerine conveyed the disease after standing 24 hours, but not after 72 hours. A mixture of the same emulsion with an equal volume of 2 per cent trikresol solution (making a 1 per cent mixture) was found free from the infecting agent after 2 minutes. No inoculations were made after a shorter exposure.

EXAMINATION OF STAINED SMEAR PREPARATIONS.

It was the absence of pest-like organisms in the first inoculated animal dead of this disease that led us to suspect that we were not dealing with plague. This is mentioned to illustrate the similarity of the lesions to plague, and the dissimilarity of stained smear preparations. Carbol-thionin, Loeffler's Alkaline Methylene Blue, Wright's stain and Giemsa's stain all usually fail to show the presence of anything that can be recognized as a micro-organism. The Ziehl-Nielson method likewise fails to show any organism. In many cases the smears show, especially in the leucocytes, fine dust-like objects which are not commonly seen in smears from the tissues of animals dead from other causes, although sufficient control work has not been done. At present we do not attach much significance to these objects, but they will be investigated more fully. Numerous very small, solid staining bacilli have been seen on a number of occasions. We can not say at present what importance should be attached to them.

CULTURAL INVESTIGATIONS.

All attempts to cultivate on artificial media the organism that causes this disease have been unsuccessful. In the great majority of cases the media remained sterile, or contained growths of what were obviously contaminating organisms. On several occasions cultures that grew on the media were used to inoculate guinea pigs, but without success in reproducing the disease. What has been said refers to aerobic cultures on agar and in broth and to liberal inoculations on the surface of agar or in broth with blood from infected squirrels and guinea pigs. Cultures in freshly steamed glucose broth in Dunham's fermentation tubes usually remained sterile, as did those in broth covered with oil. On one occasion a growth in a fermentation tube showed in smears rods and cocci. Some of the fluid from the tube was used to inoculate a guinea pig, with the result that the animal died of the disease on the ninth day. Cultures made from the guinea pig remained sterile. As the original fermentation tube had been heavily inoculated with the blood, we were inclined to attribute the death of the guinea pig to the blood in the mixture. Attempts to grow the causative organism are still being made.

FILTRATION EXPERIMENTS.

Several experiments have been made for the purpose of determining whether the cause of the disease was an organism small enough to pass through the so-called "bacteria proof" filters. The writer's experience with this class of work is so limited that he would hesitate

to report any experiments tending to show that an organism was so small as to pass through one of these filters, and consequently small enough to raise a question as to whether it might belong to the class known as ultra-microscopic organisms. However, every experiment showed that the causative agent did not pass through the Berkefeld filter. In each case control animals died of the disease in the usual time, while those inoculated with the filtrate, secured under a negative pressure of 15 pounds, remained well.

MODE OF TRANSMISSION.

Several experiments have been made for the purpose of learning the natural mode of transmission of the disease.

CONTACT.

On several occasions healthy guinea pigs and ground squirrels have been put in small cages with infected rodents, left there until the latter died and kept in the uncleaned cage for several days after the death of the sick one, but in no case was the disease transmitted even under these circumstances of intimate contact. It has been shown that feeding experiments are successful in transmitting the disease. We have good reason for believing that in nature it is not ordinarily transmitted in this manner. The reason for this is the fact that in the feeding experiments the buboes of the animals infected by ingestion were in the neck or in the mesentery, while when the disease is naturally contracted by squirrels the bubo is often situated in other parts of the body, axilla, pelvis, or inguinal regions. As the most prominent feature of the disease in most naturally infected rodents is a lesion of the peripheral lymph glands, it would seem reasonable to believe that the infection usually gains entrance through the skin. The unsuccessful contact experiments, four in number, in which healthy guinea pigs were kept with sick ones and allowed to remain in the cages, some of which were very filthy by reason of the accumulation of urine and feces from the sick and the well animals, negatived the probability that the disease was transmitted by means of contact with sick animals or their excretions.

INSECT TRANSMISSION.

It is more in keeping with our present views of the transmission of many infectious diseases to look for a living carrier, and as this disease has so many points of resemblance to plague, which has been shown to be readily transmitted among rats, guinea pigs, and squirrels by means of fleas, we naturally turned to these parasites as a probable means of conveying the disease. It was found possible to reproduce the infection in guinea pigs by subcutaneous inoculation with crushed squirrel fleas (*Ceratophyllus acutus*) taken from a squirrel or from a guinea pig dead of the disease. The successful

experiments were performed by inoculating the guinea pigs with fleas not more than 24 hours after removing them from recently dead rodents. The infection of the guinea pig in this manner proves nothing more than that the rodent from which the fleas were taken had a septicæmia. In one case in which 10 fleas were kept (alive) from 3 to 5 days before injecting them into guinea pigs, the animals remained well.

Attempts were made to infect guinea pigs and squirrels by placing the fleas, which had been removed from rodents recently dead of the disease, in clean cages with the healthy animals, and also by placing healthy rodents (squirrels and guinea pigs) in cages from which a squirrel dead of the disease had been removed. Large numbers of fleas (*C. acutus*) were present, but in no case did the animal contract the disease.

In two cases in which healthy squirrels were introduced into cages with sick animals and in the presence of large numbers of squirrel fleas (*C. acutus*) infection of the healthy rodents occurred; while in adjacent cages in which all conditions were the same, but no fleas present, the healthy animals remained unaffected.

Flea transfer No. 1 (November 11, 1910).—A squirrel was inoculated cutaneously with spleen from a naturally infected squirrel. Two healthy squirrels were placed in the cage with it and 100 fleas (*C. acutus*). The inoculated animal died on the sixth day. One of the contacts died on the nineteenth day with the usual lesions of the disease, the bubo being in the median inguinal region; the other died on the twenty-eighth day with a bubo in the axilla and other characteristic findings.

Flea transfer No. 2 (October 12, 1910).—A squirrel was vaccinated with the liver of a guinea pig dead of the disease. Two healthy squirrels were placed in the cage with this animal, together with about 500 squirrel fleas (*C. acutus*). The inoculated rodent died with the usual lesions 5 days after inoculation. The other squirrels both died, one 4 days and the other 7 days after the death of the inoculated one. In one the bubo was in the mesentery, in the other it was in the neck. On account of the location of the buboes, we were inclined to attribute the infection in these cases to ingestion rather than to flea transference.

GENERAL REMARKS.

The disease appears to be uniformly fatal to squirrels, guinea pigs, and mice. Although but few squirrels and mice were used, every one that was inoculated succumbed. Of the large number of guinea pigs used, but one survived the inoculation, and we are in doubt whether this was an example of defective technique, the absence of the infectious agent from the material used (vaccination with heart's

blood from a guinea pig), or of a natural resistance. The disease has not been observed to follow a chronic course in guinea pigs, mice, or squirrels. The time of death and the lesions indicate that the process is an acute one. The following table shows the day of death of guinea pigs inoculated by various methods:

Day of death.	Inoculation.			
	Vaccination.	Subcutaneous.	Nasal.	Intraperitoneal.
First	0	0	0	0
Second	0	0	0	0
Third	0	1	0	1
Fourth	2	1	0	0
Fifth	11	8	0	0
Sixth	21	14	2	0
Seventh	18	10	0	0
Eighth	11	1	0	0
Ninth	3	0	0	0
Tenth	1	1	0	0
Eleventh	2	1	0	0
Twelfth	1			

The virus has been carried through eleven generations in guinea pigs without any evidence of loss of virulence.

We do not know whether the organism causing this disease is pathogenic for man, but judging from the large number of species that are susceptible, we are inclined to suspect that man might contract the infection.

SUMMARY.

A disease which presents lesions very similar to those of plague has been found among ground squirrels.

The disease is readily transmitted to guinea pigs, mice, rabbits, monkeys, and gophers, and plague-like lesions are produced in at least some of these animals. Rats are but moderately susceptible to the infection. Cats, dogs, and pigeons appear to be immune.

The disease may be transmitted artificially by subcutaneous, cutaneous (vaccination), nasal, and intraperitoneal inoculation. The mode of transmission in nature is unknown, but there is some experimental evidence that suggests that fleas may serve as carriers. The disease probably is not spread by mere contact of healthy with infected animals.

The infectious agent has not been isolated. On account of the number of species susceptible, it seems likely that it is bacterial, not protozoan.

The causative agent is present in the circulating blood, as well as in the various tissues where it causes focal lesions.

The thermal death point of the organism as it is found in the animal's body is between 55° C. and 60° C.

Several observations appear to indicate that the disease is a febrile one.

LIST OF PUBLIC HEALTH BULLETINS.

The following is a list of the Public Health Bulletins that have been issued:

*1. Report on Trichinæ and Trichinosis. By W. C. W. Glazier. 1881. 212 pages. 87 il. 1 map. Paper. Senate Executive Document No. 9, Forty-sixth Congress, third session. Out of print.

*2. Report on the Etiology and Prevention of Yellow Fever. By George M. Sternberg. 1890. 271 pages. 21 pl. 20 il. Cloth. Out of print.

3. Mortality Statistics in the United States for the year ending December 31, 1897. From Annual Report Marine-Hospital Service, 1898. 24 pages. Paper.

4. Yellow Fever: Its Nature, Diagnosis, Treatment, and Prophylaxis and Quarantine Regulations Relating thereto. By officers of the Marine-Hospital Service. Reprint from Annual Report Marine-Hospital Service, 1898. 176 pages. 1 il. Paper.

*5. Shipment of Merchandise from a Town Infected with Yellow Fever. By H. R. Carter. 1899. 15 pages. Paper. Out of print.

6. Report of Commission of Medical Officers detailed by authority of the President to Investigate the Cause of Yellow Fever. By Eugene Wasdin and H. D. Geddings. July, 1899. 98 pages. 26 charts. 2 il. Paper.

*7. The Bubonic Plague. By Walter Wyman. January, 1900. 50 pages. Paper. Superintendent of Documents, 5 cents.

*8. Report of Commission appointed by the Secretary of the Treasury for the Investigation of Plague in San Francisco. By Prof. Simon Flexner, Prof. F. G. Novy, and Prof. L. F. Barker. January 23, 1901. 23 pages. 1 map. Paper. Out of print.

*9. Report Relating to the Origin and Prevalence of Leprosy in the United States. By a commission of Medical Officers of the U. S. Marine-Hospital Service. 1902. 119 pages. 25 il. Paper. Senate Document No. 269, Fifty-seventh Congress, first session. Superintendent of Documents. Cloth, $1.00.

*10. Plague Conference. Containing a copy of the address of the chairman, and resolutions passed by a conference called in accordance with requests from a number of State Boards of Health, and under authority of section 7, act of Congress approved July 1, 1902, to consider the plague situation. Reprint from P. H. R. No. 4, Vol. XVIII, January 23, 1903. 9 pages. And February 6, 1903. 41 pages. Paper. Out of print.

11. Transactions of the First Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. June, 1903. 120 pages. Cloth.

12. Transactions of the Second Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. June, 1904. 95 pages. Cloth.

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* Exhausted and not for distribution.

13. The Public Health and Marine-Hospital Service of the United States. A Brief History. Prepared for the Louisiana Purchase Exposition. December, 1904. 16 pages. Paper.

*14. Sanatorium for Consumptives, Fort Stanton, N. Mex. By P. M. Carrington. Reprint from Annual Report Public Health and Marine-Hospital Service, 1904. 19 pages. Paper. Out of print.

15. Transactions of the Third Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. May, 1905. 52 pages. Cloth.

16. How to Prevent Yellow Fever—No Mosquitoes, No Yellow Fever. By Walter Wyman. July 31, 1905. 3 pages. Circular.

17. Transactions of the Fourth Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. May, 1906. 75 pages. Cloth.

18. Transactions of the Fifth Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. May, 1907. 47 pages. Cloth.

19. Trachoma, Its Character and Effects. By Taliaferro Clark and J. W. Schereschewsky. 1907. 34 pages. 6 il. Paper.

*20. The Public Health and Marine-Hospital Service of the United States. A Brief History. Prepared for the Jamestown Ter-Centennial Exposition. 1907. 12 pages. Paper. Out of print.

21. Transactions of the Sixth Annual Conference of State and Territorial Health Officers with the United States Public Health and Marine-Hospital Service. April, 1908. 79 pages. Cloth.

*22. The Present Pandemic of Plague. By J. M. Eager. 1908. 30 pages. Paper. Out of print.

23. Pellagra—A Precis. By C. H. Lavinder. July 24, 1908. 22 pages. 1 il. Paper.

24. The Marine-Hospital Sanatorium, Fort Stanton, N. Mex. Prepared for the International Congress on Tuberculosis, held in Washington, September, 1908. 32 il. 56 pages. Paper.

*25. Hookworm Disease. Reprint from Annual Report P. H. and M. H. S., 1908. 5 pages. Paper. Out of print.

26. Studies upon Leprosy.
    I. The Present Status of the Leprosy Problem in Hawaii.
    II. The Reaction of Lepers to Moro's "Percutaneous" Test.
    III. A Note Upon the Possibility of the Mosquito Acting in the Transmission of Leprosy. By W. R. Brinckerhoff. 1908. Investigations made in accordance with the Act of Congress approved March 3, 1905. 24 pages. Paper.

27. Studies upon Leprosy.
    IV. Upon the Utility of the Examination of the Nose and the Nasal Secretions for the Detection of Incipient Cases of Leprosy. By W. R. Brinckerhoff and W. L. Moore. 1909. Investigations made in accordance with the Act of Congress approved March 3, 1905. 29 pages. Paper.

28. Studies upon Leprosy.
    V. A Report upon the Treatment of Six Cases of Leprosy with Nastine (Deycke). By W. R. Brinckerhoff and J. T. Wayson, Honolulu, T. H.

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* Exhausted and not for distribution.

28. Studies upon Leprosy—Continued.
    VI. Leprosy in the United States of America in 1909. By W. R. Brinckerhoff. 1909. Investigations made in accordance with the Act of Congress approved March 3, 1905. 25 pages. Paper.
29. The Prevalence of Rabies in the United States. By J. W. Kerr and A. M. Stimson. 1909. 16 pages. Paper.
30. The Rat and its Relation to the Public Health. By various authors. 1910. 254 pages. 60 figs. 6 pls. Paper.
    1. Introduction. By Walter Wyman.
    2. Natural History of the Rat. By D. E. Lantz.
    3. Plague Infection in Rats. By G. W. McCoy.
    4. Rat Leprosy. By. W. R. Brinckerhoff.
    5. Bacterial Diseases of the Rat other than Plague. By D. H. Currie.
    6. Organic Diseases of the Rat. By G. W. McCoy.
    7. Ecto Parasites of the Rat. By N. Banks.
    8. Intestinal Parasites of Rats and Mice in their Relation to Diseases of Man. By C. W. Stiles.
    9. Rodents in Relation to the Transmission of Bubonic Plague. By Rupert Blue.
    10. Rodent Extermination. Rats and Mice. By W. C. Rucker.
    11. Natural Enemies of Rats. By D. E. Lantz.
    12. Rat-Proofing as an Antiplague Measure. By R. H. Creel.
    13. Inefficiency of Bacterial Viruses in the Extermination of Rats. By M. J. Rosenau.
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FIG. 1.—BUBO; ACUTE PLAGUE IN GROUND SQUIRREL.

FIG. 2.—SPLEEN; SUBACUTE PLAGUE IN GROUND SQUIRREL, SMALL CASEOUS FOCI.

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FIG. 3.—SPLEEN; SUBACUTE PLAGUE IN GROUND SQUIRREL, LARGE AND SMALL CASEOUS FOCI.

FIG. 4.—SPLEEN; SUBACUTE PLAGUE IN GROUND SQUIRREL, SINGLE LARGE ABSCESS.

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FIG. 5.—SUBACUTE PLAGUE IN GROUND SQUIRREL, DIFFUSE CONSOLIDATION OF LUNGS.

FIG. 6.—SUBACUTE PLAGUE IN GROUND SQUIRREL, LARGE AND SMALL ABSCESSES IN LUNGS.

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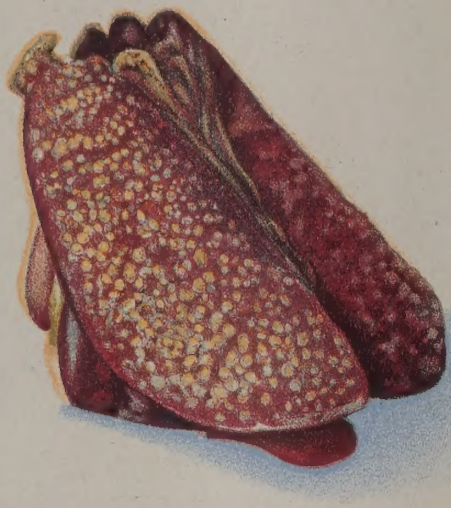

FIG. 7.—SUBACUTE PLAGUE IN GROUND SQUIRREL, SMALL ABSCESSES IN LUNGS.

FIG. 8.—RESIDUAL PLAGUE BUBO.

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FIG. 9.—BUBO; PLAGUE-LIKE DISEASE IN GUINEA PIG.

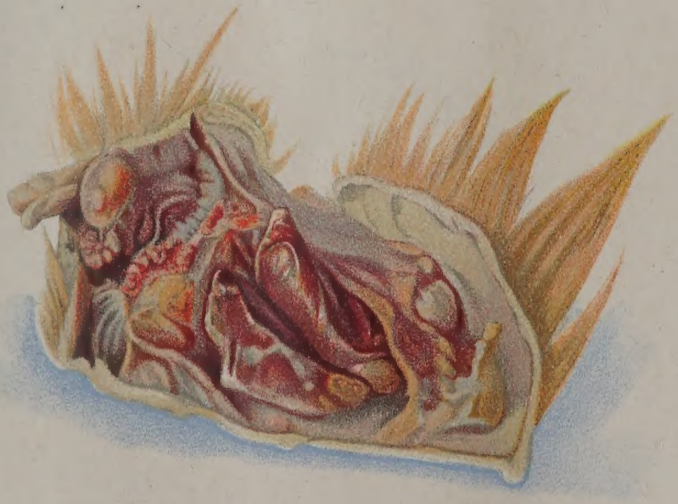

FIG. 10.—BUBO; PLAGUE IN GUINEA PIG.

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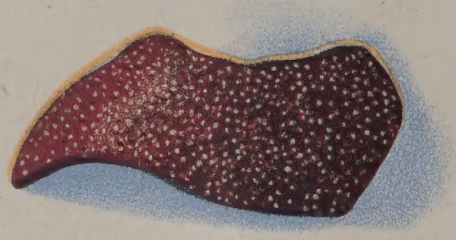

Fig. 11.—Spleen of Squirrel, Plague-like Disease.

Fig. 12.—Liver of Guinea Pig, Plague-like Disease.

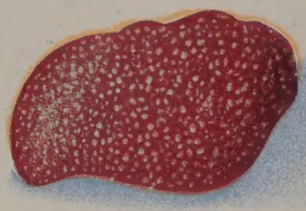

Fig. 13.—Spleen of Guinea Pig, Plague-like Disease.

Fig. 14.—Liver of Guinea Pig, Plague.

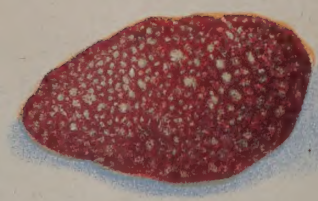

Fig. 15.—Spleen, Plague in Guinea Pig.

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